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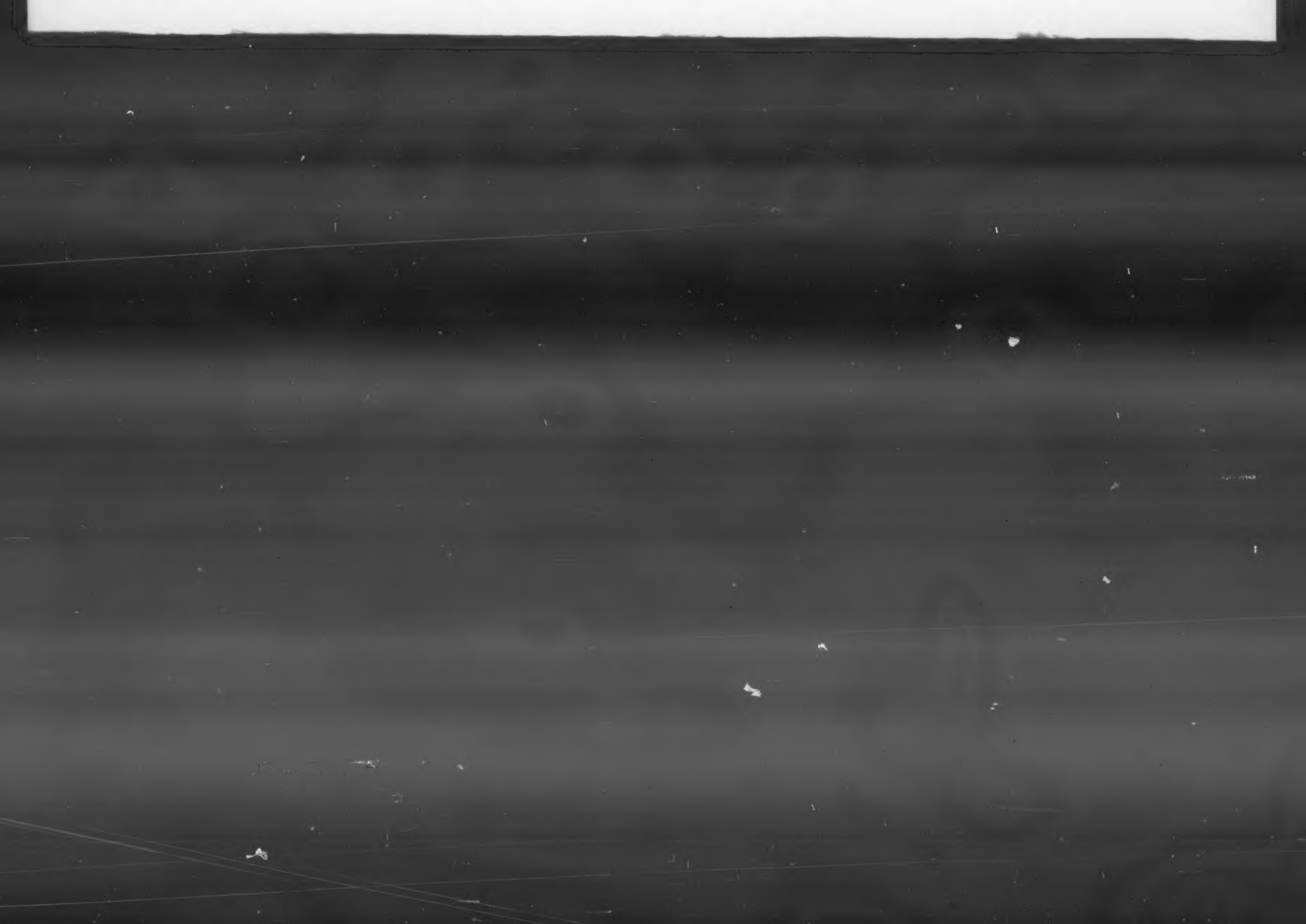

ACTA PHYSIOLOGICA LATINOAMERICANA

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THE EFFECTS OF SEXUAL GLANDS AND STEROIDS ON DIABETES IN PARTIALLY PANCREATECTOMIZED RATS

RICARDO R. RODRÍGUEZ

(Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires, Argentina)

WITH the modification of Foglia ([5]) of the Shapiro and Pincus ([2]) technique for extirpation of 95 per cent of the pancreas in the rat, it is possible to produce an experimental diabetes in this animal. The diabetes observed in the rat has many advantages from the experimental point of view over that provoked in other animals: a) it is possible to work with a standard animal, because uniform genetic strains can be obtained by inbreeding; b) the ease and low price of breeding of these animals allow the use of great numbers; c) they are omnivorus; d) they have a short life span, etc.

Foglia ([5]) has made a detailed study of the evolution of the diabetes in rats after ablation of 95 per cent of the pancreas. The course of the disease may be divided into three periods: 1) a prediabetic stage which lasts several weeks when the animals are apparently normal; the fasting blood sugar and the glucose tolerance curves are normal, and no glycosuria is found; 2) incipient diabetic stage with normal fasting blood sugar, diabetic glucose tolerance curve and post-absorptive glycosuria; and, 3), diabetic stage in which a fasting blood sugar more than 150 mg per cent and a fasting glycosuria is present. This type of experimental diabetes which can be controlled by diet and insulin resembles human diabetes more closely in its characteristics than other forms of experimental diabetes.

Using this technique, Foglia ([6]) found a sex difference in relation to the onset and incidence of diabetes in rats of the same age; the incidence of diabetes being higher and the time of onset shorter in male than in female rats.

In this paper we summarize our studies on the mechanism of this sexual difference, showing that hormones have a definite effect on the incidence of diabetes in the subtotally pancreatectomized rats and in their subsequent well-being.

We are indebted to Syntex, Organon, Schering Corporation, Richardson, Estrona, Ciba, Glaxo, Squibb Laboratories and Corn Refineries for generous supplies of materials used in these studies.

SEXUAL DIFFERENCE

When pancreatectomy was carried out by the same operator, in normal male and female rats of the same age and receiving the same diet *ad libitum* (for composition, see table I), a sexual difference was evident. The series of males and females were operated on and experiments carried out at the same time to assure the uniformity of the experimental conditions for both sexes. Table II shows that frequency of diabetes is much higher in males than in females.

A more detailed study (chart 1) shows that the incidence of diabetes in females increase gradually in a straight line. In males there is an abrupt increase during the 2nd and 3rd months and the maximum is reached at the 6th month.

TABLE I

Composition of ad libitum diet.

Per cent		Carbohydrate	Protein	Fat	Water	Cellulose	Calories
Milk	64	3.07	1.98	1.98	55.68	—	33.80
White bread	20	11.48	1.85	0.03	6.17	0.04	53.70
Wheat	8	6.09	0.84	0.11	0.64	0.13	28.75
Corn	8	5.31	1.01	0.29	0.76	1.60	27.97
Total	100	25.95	5.68	2.41	63.25	1.77	149.22

TABLE II

Sex difference on the incidence of diabetes in subtotally pancreatectomized rats, six months after operation (Rodríguez, 1950).

	Number of rats operated	Number of diabetics	Percentage
Male rats	140	125	89
Female rats	120	34	27

Sexual difference and diet.

Male rats of 170 g body weight eat daily an average of 27 g of a standard diet; female rats of the same weight eat only 21 g of the same

standard diet. Over-feeding produces diabetes and underfeeding has a protective effect ([23]). Therefore, the influence of paired feeding and forced feeding on the appearance of diabetes was investigated.

(A) *Paired-feeding experiments.* — Rats of both sexes and of the same weight were studied in pairs (Foglia, Schuster and Rodríguez, [8, 9]); the food ingested by the female was weighed and the same amount was then given to the male on the next day. Sixteen such paired-feeding experiments in pancreatectomized animals were carried out and table III and chart 2 shows that even under these conditions the incidence of diabetes was higher in males.

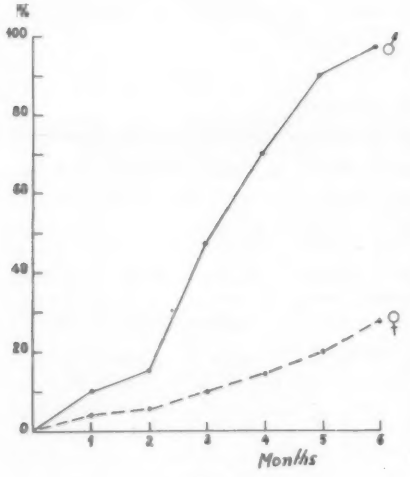

Chart 1

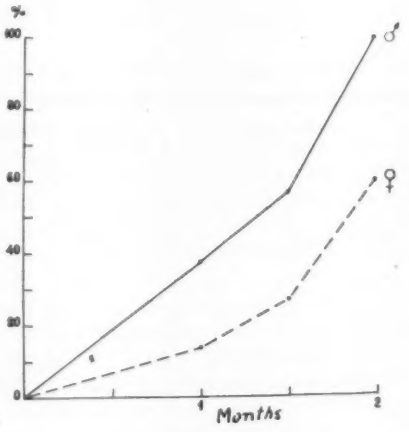

Chart. 2

CHART 1. — *Incidence of diabetes (values per cent) in 66 male and 97 female rats observed monthly after subtotal pancreatectomy (Foglia, Schuster and Rodríguez, 1947).*

CHART 2. — *Sex difference on the incidence of diabetes in 95 % pancreatectomized rats with paired feeding (16 pairs) (Foglia, Schuster and Rodríguez, 1947).*

(B) *Forced-feeding experiments.* — The diet used had the composition shown in table IV.

The animals received a total daily dose of 25 ml, divided in 2 rations. They were fed from 8 to 8.45 a.m., and from 4.30 to 5.30 p.m. The mixture was warmed up to 35°C, immediately before feeding. It was observed that the animals remained for 2 or 3 hours in a state of sopor after the feeding. In order to avoid alimentary shock, a total daily ration of 10 ml was given at the beginning, increasing it 1 ml daily, in such a manner that 2 weeks later the animals were receiving the full daily dose of 25 ml.

TABLE III

Incidence of diabetes in male and female 95 % pancreatectomized rats with paired feeding (average of 15 females and 16 males). (Foglia, Schuster and Rodríguez, 1947).

Months	Body weight g		Average fasting blood sugar of diabetics mg %		Incidence of diabetes %	
	Males	Females	Males	Females	Males	Females
0	88	89	108	104	0	0
1	157	148	157	154	38	13
1 ½	—	—	180	171	56	27
2	190	178	200	177	100	60
2 ½	—	—	187	180	—	—

TABLE IV

Composition of forced fed diet

Cellu-flour	120
Salt mixture (McCollum-Davis)	40
Yeast	100
Dry milk	600
Wheat germ oil	12
Mazola oil	220
Cod liver oil	12
Water	1400

The final % composition of the diet was:

Water	53.00
Dry residue	47.00
Carbohydrate	15.00
Protein	8.30
Fat	17.35
Cellulose	3.00
Ash	3.35

Results of this experiment are shown in chart 3. The incidence of diabetes was again higher in males.

These results seem to exclude an alimentary factor as the exclusive or principal cause of the sexual difference in diabetes.

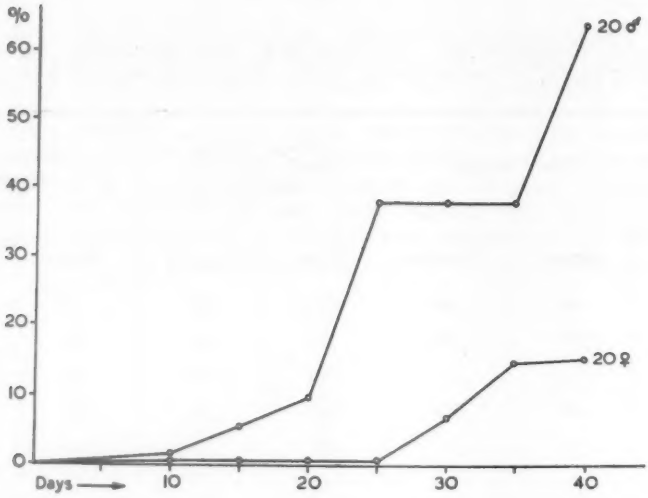

CHART 3. — *Influence of forced feeding on the incidence of diabetes in partial pancreatectomized rats.*

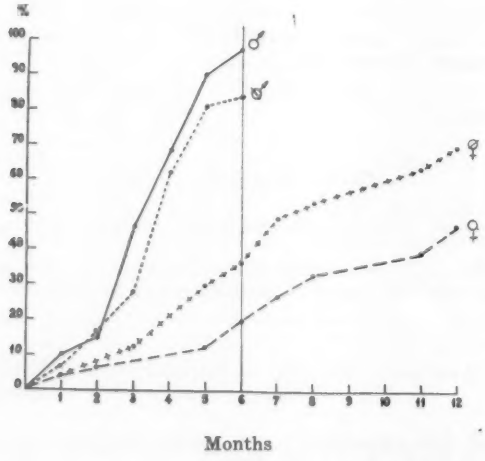

CHART 4. — *Influence of castration on the incidence of diabetes after 95 % pancreatectomy (66 males, 32 castrated males, 30 females and 35 castrated females) (Foglia, Schuster and Rodríguez, 1947).*

INFLUENCE OF GONADS

After discarding alimentary factors as the sole cause of the sexual difference, the influence of the gonads was studied. The testes might sensitize the rat to diabetes or the ovaries might have a protective action.

In male rats simultaneous castration reduces slightly the incidence of diabetes by pancreatectomy (chart 4). If castration is effected after the onset of diabetes, it produces no difference in rats [30, 31] or in dogs [26].

On the contrary, the incidence of diabetes was higher in the ovariectomized and pancreatectomized rats, than in the controls with only pancreatectomy. The difference appeared one month after the operation and was considerable after one year (chart 4).

As shown in chart 4, the curves after castration in males and females tend to approximate each other but do not coincide. The operation was performed in these rats when they weighed 70 grams; perhaps in rats castrated at birth the difference would be less or would disappear entirely.

ACTION OF STEROIDS

There are contradictory reports on the effect of estrogens on diabetes. Thus, according to one group of workers, the severity of diabetes is diminished by treatment with estrogens in the dog [1] and in the monkey [24, 25]. On the other hand, certain workers have not observed any effects in the dog [32] or in the monkey [3]. In the ferret, estrogens have been reported to increase the severity of diabetes [4].

In forced-fed animals, Ingle [14, 15] and Ingle and Nezamis [19] observed the appearance of glycosuria in normal rats, or its increase if already present, in partially pancreatectomized rats, under administration of diethylstilbestrol. This substance had no effect on the glycemia or glycosuria of partially pancreatectomized male rats, providing the animals were allowed to eat *ad libitum* [20, 18].

The next experiments show the action of different steroids in *ad libitum* and forced feeding subtotal pancreatectomized rats.

A) *Action of various steroids in ad libitum-fed rats.* — In this series of experiments (Lewis, Foglia and Rodríguez, [24]), white rats were castrated when they had reached 80 to 100 g of body weight. A week later 95 per cent of the pancreas was removed following Foglia's technique. Each lot was operated on by the same person at the same time, and was afterwards divided into groups receiving treatment with one or other steroid, and controls injected with lactose; thus differences due to removal of a varying amount of pancreas were reduced to a minimum. A week after pancreatectomy, treatment was commenced, consisting of one daily subcutaneous injection of the steroid mixed with 25 mg of lactose suspended in 0.5 ml saline (0.9 % NaCl) solution. The controls received 25 mg lactose in saline. The treatment was continued for six months.

The body weight and blood sugar were determined monthly, and an animal was considered to be diabetic when the blood sugar was above 150 mg per cent after a 7 hour fast.

TABLE V

Effect of different substances injected daily during 6 months on the incidence of diabetes in male and female castrated rats, after removal of 95 per cent of the pancreas (Lewis, Foglia and Rodríguez, 1949-50; Rodríguez, 1950).

	Daily dose per rat μg	Castrated females				Castrated males	
		exp. A-D		exp. B-C			
		Diabetics	Percentage	Diabetics	Percentage	Diabetics	Percentage
Lactose	25 (mg)	12/23	52	26/35	74	16/18	89
Cholesterol	500	4/15	27	5/9	55		
Estrone	15	3/10	30				
Estrone	50	0/8	0				
Diethylstilbestrol	15	3/10	30				
Diethylstilbestrol	50			1/8	12	3/16	50
Phenocycline	15	0/9	0				
Phenocycline	50	1/11	9				
Dienestrol				1/15	6		
Ethynil-estradiol	50			4/16	25		
Ethynil-testost.	500			4/9	44		
Equilenine	50	6/8	75				
Progesterone	500			6/9	66		
Desoxycorticost	100			8/12	66		
Testosterone	50	11/15	73			13/14	92
Testosterone	500	8/8	100				
Methyl-Testost.	500			9/11	83		
17 β-ethyl-dihydro testosterone	500			8/11	72		

In the forced-feeding experiments, each animal was kept separately in a single cage, at a temperature of 22° to 23°C. They drank water at will. The urine of each animal was collected in the usual manner, preserved under toluol and every 48 hours determination of its glucose content was made by the Benedict method. Blood samples were drawn from the tail 7 hours after the morning forced-feeding. Glycemia was determined, in all the cases, by the Hagedorn and Jensen method.

At the end of the experiment the animals were killed, the organs weighed and the pancreas, adrenals and hypophysis fixed for histologic study.

Relation between islets of Langerhans and pancreatic acini. — Was performed by Dr. A. F. Cardeza. The pancreas was fixed in Bouin-Hollande solution and paraffin section of 4 microns were made and stained with hematoxylin-eosin. Six different parts of each preparation were projected by camera lucida with a magnification of 70 diameters onto a white paper of uniform thickness and an outline was made of the islets and the insular tissue. The islets and acini sketchs were cut out and weighed separately with precision in mg. From this data the following ratio was calculated: $\frac{weight\ islets \times 100}{weight\ acini}$

The results of each series are given in tables V, VI and VII and charts 5 and 6.

TABLE VI

Effect of estradiol benzoate injected daily during 6 months on the incidence of diabetes in intact and castrated male and female rats after removal of 95 % of the pancreas (Foglia, Schuster and Rodríguez, 1947).

Daily dose per rat μg	Intact males				Castrated males			
	Controls		Treated		Controls		Treated	
	Diabetic	Percentage	Diabetic	Percentage	Diabetic	Percentage	Diabetic	Percentage
2	11/12	92	4/11	36	8/13	61	4/11	36
4	8/9	88	4/8	50	7/8	100	4/9	44
15	10/10	100	2/12	16	8/8	87	6/9	66
	Intact females				Castrated females			
2	6/14	43	1/12	8	2/9	22	2/12	16
4	13/63	21	—	—	19/52	37	1/13	8
15	5/7	71	2/6	33	6/7	86	1/8	12

Estrone. — This substance exerted a protective action in doses of 15 and 50 μg daily, the effect being more marked with the larger dose. The hair on the caudal part of the back and on the abdomen was lost almost completely. There was a slight fall in body weight and in the weight of the thymus. The hypophysis, adrenals, thyroid, uterus and kidney were enlarged.

Stilbestrol (dipropionate). — Daily doses of 15 and 50 μg had a protective action on castrates of both sexes, which was more marked with the higher dose. There was considerable loss of hair all over the body in most animals and a marked fall in body weight. The hypophysis was found to be greatly enlarged; the adrenals were much above the normal weight, and the uterus, kidneys and liver were also enlarged. The prostate and seminal vesicles were very large although the animals were castrated; in one case hypertrophy of the prostate caused retention of urine in the bladder and the animal died. The thyroid showed no change in weight and the thymus a moderate atrophy.

TABLE VII

Ratio between pancreatic islets and acini of non diabetic castrated female rats, with subtotal pancreatectomy, injected with steroids during 6 months. Controls with lactose (Cardeza, 1950).

Substance	Number of animals	Weight islets × 100 / weight acini
Lactose	4	1.66
Ethynil-estradiol	2	6.94
Diethylstilbestrol	4	6.93
Estrone	2	6.77
Dienestrol	2	6.18
Estradiol	3	5.42
Ethynil-testosterone	1	2.98
Progesterone	1	1.84

Dienestrol. — Doses of 50 μg daily had a protective effect. The general condition of the treated animals was good, although the body weight was slightly below that of the control. There was also some fall of hair on the caudal part of the back and the abdomen. The hypophysis, adrenals, uterus, kidneys and liver were enlarged; the thyroid and thymus were of normal weight.

Estradiol (benzoate). — This substance had a protective action when injected in doses of 2, 4, and 15 μg in intact and castrated animals of both sexes. The results are summarized in table VI and chart 5.

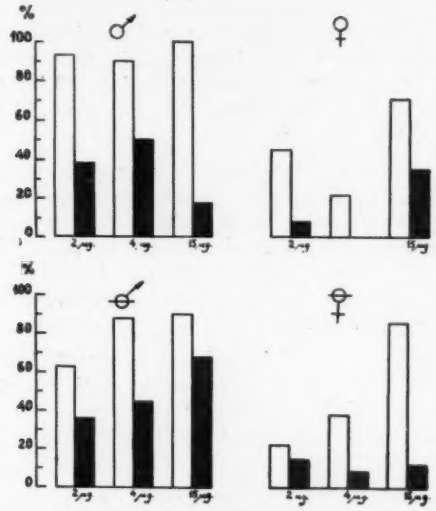

CHART 5. — *Influence of injection of estradiol benzoate on the incidence of diabetes after 95 % pancreatectomy. Black columns: injected; white columns: controls (Foglia, Schuster and Rodríguez, 1947).*

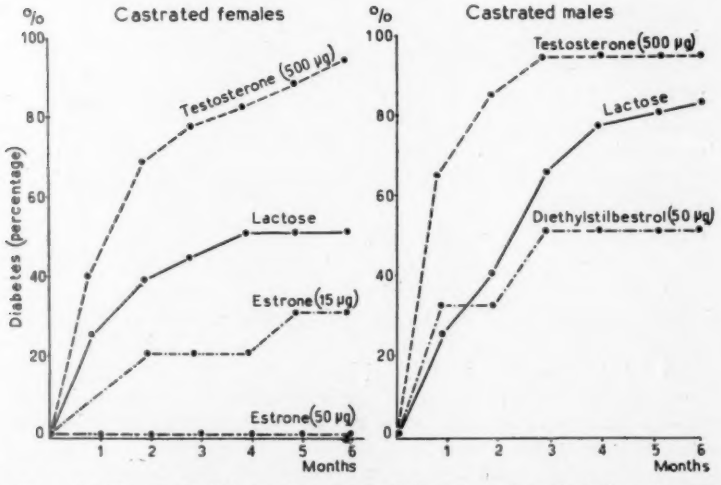

CHART 6. — *Incidence of diabetes in castrated female and castrated male rats, with subtotal pancreatectomy, injected daily during six months with estrogens and androgens (controls with lactose); daily dose per rat in micrograms (Lewis, Foglia and Rodríguez, 1949-50).*

Fenocyclin (L-methyl-bisdehydrodoisynolic acid). — In the castrate female rat with subtotal pancreatectomy, daily doses of 15 and 50 µg had a marked protective action. The body weight was much lower than that of the controls and there was considerable loss of hair. The hypophysis, adrenals, thyroid, uterus, and kidneys were enlarged and the weight of the thymus was below the normal.

Ethynil-estradiol. — A daily dose of 50 µg decreased the incidence of diabetes. The body weight was much lower than that of the controls and there was considerable loss of hair; at the end of the six months' treatment the animals were almost completely bald. The enlargement of the hypophysis and adrenals was as great as in rats treated with stilbestrol. The uterus, liver and kidneys were also enlarged. The thymus and thyroid were of normal weight.

Ethynil-testosterone. — This substance exerted a protective effect at doses of 500 µg per day. The general condition of the animals was good and their body weight the same as that of the controls. There were no significant differences in the weight of the organs with respect to that of the controls.

Equilenine. — There was no protective effect on the incidence and course of diabetes in animals treated with 50 µg daily. There was some fall of hair and the body weight was slightly lower than that of the controls. The hypophysis, adrenals, uterus and kidneys were enlarged, the thymus atrophied, and the thyroid was of normal weight.

Progesterone. — A dose of 500 µg per day had no effect on the incidence of diabetes. The general condition was good, but the body weight was slightly lower than that of the controls. The weights of the hypophysis, thyroid and thymus were normal, the adrenals, uterus, liver and kidneys were slightly enlarged.

Desoxycorticosterone (acetate). — Doses of 100 µg per day had no effect on the incidence of diabetes. The general condition was good and the body weight was similar to that of the controls. Except for a slight enlargement of the uterus there were no significant variations in the weight of the organs.

Testosterone (propionate). — Doses of 500 µg daily increased the incidence and accelerated the onset of diabetes in both sexes. This was specially marked in the castrated males; at the end of the second month 3 out of 15 (21 per cent) of the controls receiving lactose and 12 out of 14 (85 per cent) of those receiving testosterone were diabetic.

The increase in body weight of both sexes was below that observed in the controls, the difference being specially marked in the castrated males. The hair did not fall but became rough and dirty after 1 to 2 months' treatment. The males had the penis in continuous semierection. The liver, kidneys, and genital organs (uterus in the female, prostate and seminal vesicles in the male) were enlarged; the thyroid was of normal weight, and the thymus was considerably atrophied; the hypophysis and adrenals weighed slightly less than in the controls.

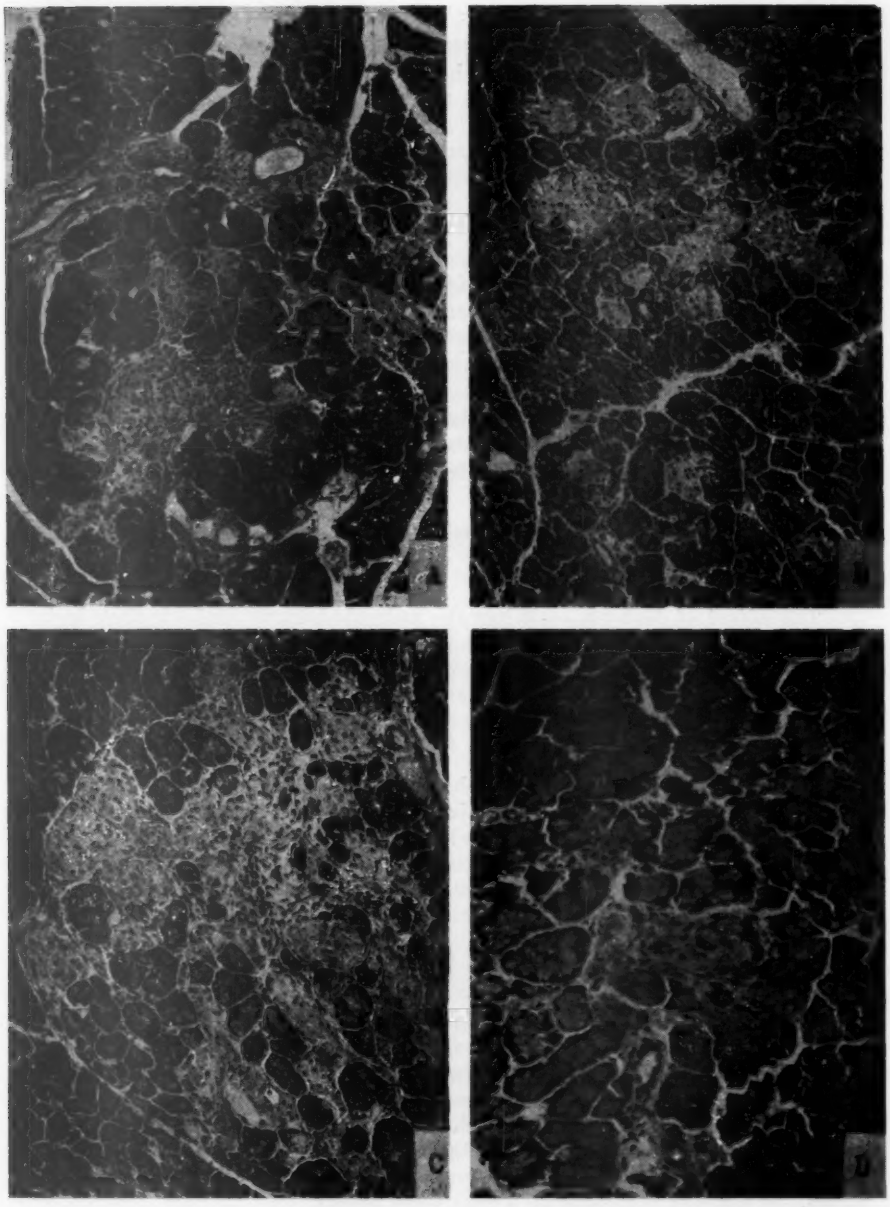

FIG. 1. — *Pancreas of partially pancreatectomized rats injected daily during 6 months with: A) lactose (nondiabetic), showing sclerosis inside and outside the islets of Langerhans; B) ethynil-estradiol (nondiabetic), showing an increased number of islets; C) diethylstilbestrol (nondiabetic), showing marked hypertrophy and hyperplasia of the islets; D) testosterone (diabetic), showing insular atrophy and necrotic beta cells. Hematoxylin-eosin* $\times$ *80.*

Methyltestosterone. — Daily doses of 500 μg accelerated the onset of diabetes. The body weight was below that of the controls, and the general condition was poor, although not so bad as that of animals injected with testosterone. The hypophysis was slightly atrophied. The weights of the thyroid, thymus and adrenals were normal. The liver and kidneys were enlarged and the uterus was large, congested and filled with fluid.

17-β-ethyl-dihydro-testosterone. — This substance had no effect on diabetes when given in doses of 500 μg daily. The general condition was good and the body weight similar to that of the controls. The hypophysis and adrenal were below normal weight, the uterus was slightly enlarged, and the thyroid, thymus, kidneys and liver normal.

Cholesterol. — Doses of 500 μg daily exerted a slight but definite protective effect (see table V). The body weight and the weight of the organs did not differ significantly from those in the controls.

Lactose. — Controls were injected with lactose and the results of each groups are given in the tables.

The effect of diabetes. — When the animals became diabetic they lost weight and eventually ended in cachexy. The general condition was specially poor when the drug injected exerted an unfavorable effect, as was the case with stilbestrol, ethynil-estradiol and testosterone. Complications of diabetes, such as cataract, were seen with equal frequency in diabetic animals treated with steroids as in the untreated controls.

The autopsy showed the hypophysis of diabetic animals in cachexy was smaller than that of nondiabetic animals in the same group; there was also atrophy of the thymus and enlargement of the adrenals, liver and kidneys. There were no changes in the weight of the thyroid and uterus except in cachectic animals, in which these organs were atrophied.

Histological examination of the pancreas. — The remaining pancreatic tissue was examined histologically by Dr. A. F. Cardeza. The controls treated with lactose showed sclerosis due to peri-insular growth of connective tissue with penetration between the islet cells and a variable degree of insular atrophy. In the nondiabetic controls the β cells were disminished in number, but the granules were well conserved and stained normally with chromic hematoxylin. The pancreas of diabetic animals stained with the same technique showed several changes: the granules were displaced to the periphery of the β cell, or were absent, there was vacuolization of the protoplasm, and a few cells had pycnotic nuclei. In some of the islets no β cells were found.

Animals treated with cholesterol which did not become diabetic showed moderate insular hyperplasia, and small newly formed islets; the

pancreas of diabetic animals in this series was in a similar condition to that of the diabetic controls.

Animals treated with estrone, estradiol, stilbestrol and ethynil-estradiol showed typical changes in the pancreas, consisting in marked hyperplasia of the insular tissue, with hypertrophy of the islets in some cases. In certain animals the newly formed islets occupied a whole pancreatic lobule, displacing the acini, which showed signs of damage due to compression. The aspect was that of an insular adenoma.

There was usually a single spherical or lobulated newly formed islet in a pancreatic lobule, with prolongations insinuated between the cells of the acini. In other cases the newly formed islets were gathered in clusters. There was little or no peri-insular collagenous reaction.

Frequently in the center of the acini, groups of hypertrophied cells, similar to β cells, in contact with centro-acinous cells were found. In favorable sections it was possible to see the continuity between centro-acinous cells loaded with granules and a newly formed islet, which was seen joined to the acinus by a pedicle of centro-acinal cells. These islets were formed mainly by β cells with a thin cortical layer of α cells, which sometimes was missing.

Rats which had been treated with estrogens but had become diabetic had moderate insular hyperplasia, with a marked decrease in β cells. These cells had lost their granules and showed vacuolization. Insular hyperplasia was greater in the animals treated with 50 μg of estrone daily than in those which had received only 15 μg daily. There was no correlation between the degree of hyperplasia and the dose injected in rats treated with stilbestrol.

In rats treated with ethynil-estradiol and ethynil-testosterone, which had not become diabetic, hyperplasia of the islet tissue was observed; while in those which became diabetic sclerosis and atrophy of the islets and degenerative lesions in the β cells were found.

Diabetic rats which had been treated with equilenine had sclerosis and atrophy of the islets.

Diabetic rats which had been injected with testosterone and methyl-testosterone showed marked sclerosis and atrophy of the islets. There were only α cells with one or two degranulated and vacuolized β cells. In one animal treated with methyl-testosterone which had not become diabetic there were a fair number of islets with some sclerosis but with well granulated cells.

Rats treated with progesterone, desoxycorticosterone and 17-β-ethyl-dihydro-testosterone which had not become diabetic showed moderate insular hyperplasia with collagenous fibers surrounding the islets, i.e. incipient sclerosis. Diabetic rats in this group showed sclerosis and atrophy of the islets with typical lesions in the β cells.

B) The effect of prolonged diethylstilbestrol administration on forced fed rats. — White rats were used throughout the investigation. Female rats of 130 to 150 g of body weight were castrated, followed a week later by removal of 95 % of the pancreas using Foglia's technique. Ten days later, after recovery from the two surgical procedures, forced feeding was started. After 5 weeks under the special diet (table II) the

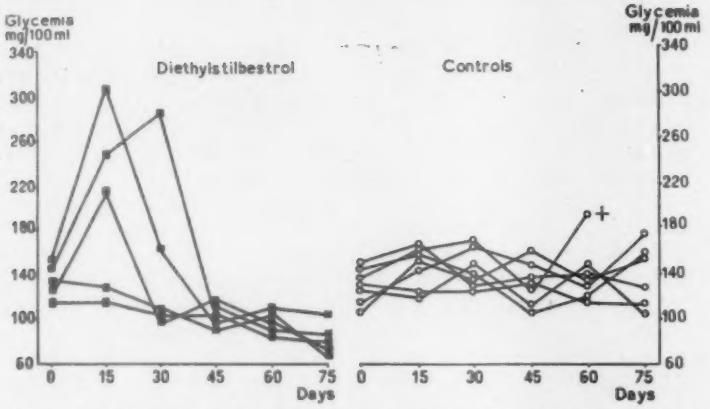

CHART 7. — *Variation of the glycemia of castrated and partially pancreatectomized female rats under forced feeding, injected with 50 μg/day of diethylstilbestrol and controls (Rodríguez, 1950).*

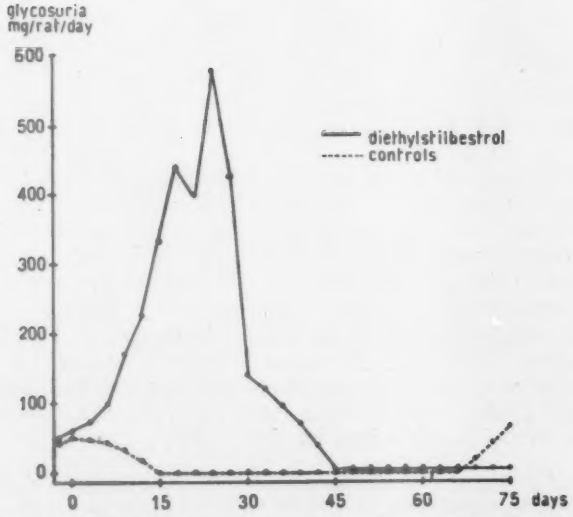

CHART 8. — *Variation of the glycosuria of castrated and partially pancreatectomized female rats under forced feeding, injected with 50 μg/day of diethylstilbestrol and controls (average of 5 rats injected and 7 controls) (Rodríguez, 1950).*

whole group was divided into two; the animals of the control group receiving 25 mg of lactose daily, subcutaneously, in 0.5 ml of 0.9 % NaCl solution; the animal of the second group being injected daily with the same dose of lactose in the saline solution and with 50 μg of diethylstilbestrol dipropionate in addition.

The data of charts 7 and 8 show that pancreatectomized rats, without hyperglycemia up to the time when diethylstilbestrol at a 50 μg daily dose was started, showed an initial rise of the glycemia and glycosuria. This increase in the glycemia and glycosuria occurred in most but not all of the animals. In the others the appearance of glycemia and glycosuria was only temporary as initial levels were resumed approximately 1 month after beginning the diethylstilbestrol treatment and while still receiving the drug. One of the nondiabetic animals died, probably due to the toxic affect of the drug.

TABLE VIII

Pituitary and adrenal weight (mg % of body weight) of castrated and 95 % pancreatectomized female rats under forced feeding. Average of 6 injected animals and 7 controls.

	Lactose	Diethylstilbestrol 50 μg/day
Hypophysis	4.7	17.9
Adrenals	28.5	46.9
Final body wt. g	234	203

At the time when diabetes appeared in the control group injected with lactose, glycosuria and blood sugar levels were normal in all the diethylstilbestrol injected animals.

Table II shows less increase of weight in the diethylstilbestrol injected animals as compared with the control series at the end of the experiment.

Autopsy findings (table VIII) of the diethylstilbestrol group showed an enlargement of the pituitary and adrenal glands.

The microscopic study of the pancreas was performed by Dr. A. F. Cardeza. Marked hyperplasia of the insular tissue was found in the diethylstilbestrol series (fig. 2 a).

Confluent masses of newly formed islets could be observed having a polylobulated and ramified appearance; transformation of the centro-acinous cells into beta cells, in different stages of the process could be seen in some small islets. Bands of sclerotic conective tissue were seen in the periphery of some insular masses. At times, these bands penetrated and cut off the beta cells trabecules. Other islets, clearly differentiated from the exocrine part did not show evidence of sclerosis; cytologically, the beta cells and their typical granules were the ones which almost exclusively could be demonstrated. The beta cells were

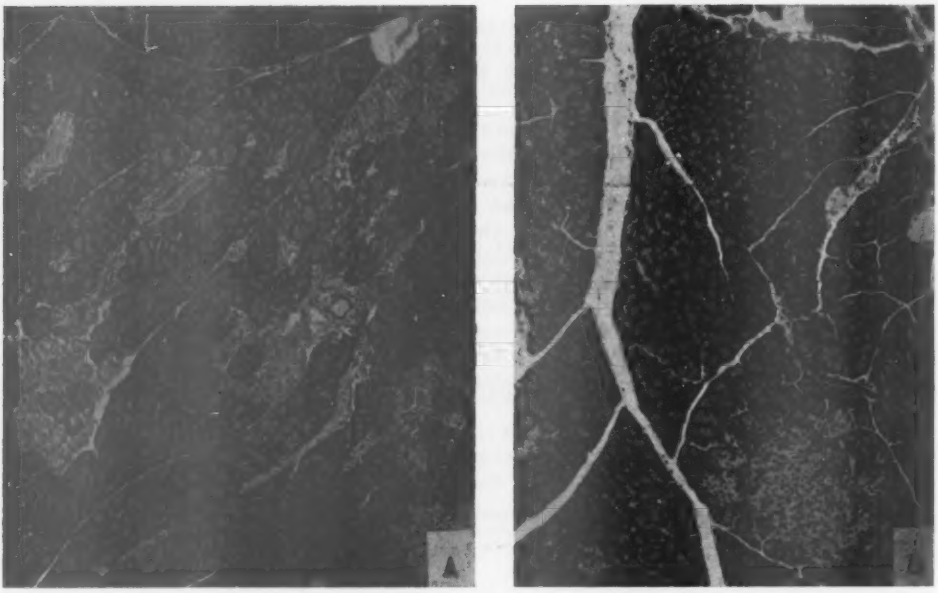

FIG. 2. — *Pancreas of nondiabetic partially pancreatectomized rats under forced feeding, injected with: A) diethylstilbestrol, showing marked hypertrophy and hyperplasia of the islets of Langerhans; B) lactose, showing sclerosis inside and outside the islets. Hematoxylin-eosin* × *60.*

hypertrophic, sometimes globulous or spherical, with a clearly visible perinuclear negative image of the Golgi apparatus when stained with Gomori's stain.

The pancreas of the nondiabetic controls (fig. 2 b), showed foci of less intense hyperplasia of the insular tissue than the animals injected with diethylstilbestrol. Collagenous tissue was present around and within the islets. The granuli of most of the beta cells were well stained. The diabetic control rats showed islets with very scant hyperplasia of the insular tissue. The beta cells showed degenerative lesions with vacuolization of the cytoplasm, nuclear atrophy and pyknosis.

The pituitary glands of the diethylstilbestrol injected rats were enlarged, with sinusoids dilatations, blood lakes and necrobiotic zones. The cytology showed an equal number of cromophobic and basophilic cells, the latter with a large intraprotoplasmic acidophilic vacuola. These elements were smaller than in the castration cells, the vacuolar content was more acidophilic and similar to the colloid substance. The pituitary gland of the control animals had the typical appearance of a castration gland, with hypertrophic basophilic cells, eccentric nuclei, the cytoplasm occupied by a large vacuola and a decrease in the eosinophi-

lic cells. Loss of hair was prominent in the diethylstilbestrol injected rats.

The increase in glycosuria observed by Ingle after diethylstilbestrol administration persists for a variable period, usually less than a month and is accompained by hyperglycemia. During this period, some animals, both with and without diabetes, died probably due to a toxic action of the drug.

Some data have been published which agree with our own findings. Ingle's figures ([14]) show that diethylstilbestrol does not maintain the increase in glycosuria initially produced by it in normal and pancreatectomized rats. The same author ([45]) later states: "When stilbestrol was administered hyperglycemia and glycosuria were induced in over two-thirds of a large series of animals studied. Daily doses as small as 50 gamma were effective. Adaptation occurred so that the diabetic state disappeared after being continually present for periods of 2 to 36 days".

After the first month of injecting diethylstilbestrol all the surviving animals remained normoglycemic and aglycosuric, a persistent condition of the treated animals even when their controls became diabetic.

THE MECHANISM OF THE ACTION OF ESTROGENS

The marked protective action of estrogens on the incidence of experimental diabetes may be due to: 1) inhibition of pituitary secretion; 2) action on the adrenal cortex; 3) direct action on the islets of Langerhans and 4) peripherial action on liver, muscle or other tissues. The following is a consideration of the first three possibilities.

1) *Action on hypophysis.*

The well-known inhibitory action of estrogens on the anterior pituitary lobe and the work of Houssay *et al.* ([12, 13]) demonstrating the action of the hormones of the *pars distalis* of this gland on carbohydrate metabolism and on diabetes, have led to the formulation of the following hypothesis: a) the rats injected with estrogens had an inhibition of the hypophysis and were similar to pancreatectomized-hypophysectomized rats, or Houssay's animals, in which the onset of diabetes was less or b) the pituitary gonadotrophins are diabetogenic and the preventive action of estrogens is due only to its inhibition.

If the first possibility were true, the hypophysetomized rats would show hypertrophy of the islets of Langerhans similar to those injected with estrogens and this is not observed ([10, 11]).

Considering the second possibility, the next experiments show that in forced-fed rats the administration of gonadotrophins has no action on the glycemia and glycosuria of subtotal pancreatectomized, diabetic animals.

Action of gonadotrophins on forced-fed diabetic rats. — Male white rats were used, from which 95 % of the pancreas was removed by Foglia's technique. When the animals became diabetic, forced feeding was

started and, five weeks later, the injections began. In the first experiment the whole group was divided into three groups: a) each rat received 20 units daily of chorionic gonadotrophin for 15 days, followed by 7 days with 100 units daily; b) all rats were injected with equal doses of serum gonadotrophin for equal lenghs of time; c) non injected control group.

In the second experiment the animals were divided into two groups: a) rats receiving 50 units of chorionic gonadotrophin for 5 days, followed by injections of 250 units daily of the same substance for 5 days; b) control rats.

TABLE IX

Influence of chorionic and serum gonadotrophins on the glycemia (mg per 100 ml) of partially pancreatectomized male diabetic rats, under forced feeding.

	Controls	Chorionic gonadotrophin	Serum gonadotrophin
Number of animals	11	12	12
Glycemia before treatment ...	166	204	188
20 u/rat/day (2 weeks)	146	186	156
100 u/rat/day (1 week)	142	172	165
1 week after injections	174	174	163

TABLE X

Variation of glycemia (mg per 100 ml) of partially pancreatectomized male diabetic rats, under forced feeding, injected with chorionic gonadotrophin; controls without injections (average of 5 animals in each group).

	Controls	Chorionic gonadotrophin
Before treatment	201	196
50 u/rat/day (5 days)	231	213
250 u/rat/day (5 days)	225	186

Results. — The data in tables IX and X shows that chorionic and seric gonadotrophins, for the time and doses given, have no significant action on the glycemia of moderately diabetic forced-fed rats. The changes in glycosuria were accompanied by similar changes in glycemia. During all of the experiments the general condition of the animals was good and no changes in body weight was noted in the injected animals.

Therefore, if the estrogens, through the hypophysis, have an influence on carbohydrate metabolism and diabetes, this action is not a simple and direct one, and there are other mechanisms concerned.

2) *Action on adrenals.*

The work of Long *et al.* ([22]) concerning the importance of the cortex of the adrenal gland on carbohydrate metabolism, and the work of Pinto ([27]) on the action of sex hormones on these glands might lead to the conclusion that the protective action of estrogens on the incidence of diabetes may be due to its action on the adrenal glands, directly or indirectly through the hypophysis.

Ingle ([16, 17]) has shown that initial rise in the glycemia and glycosuria, after diethylstilbestrol administration in pancreatectomized forced fed rats, occurred even in hypophysectomized and adrenalectomized animals, which were maintained by subdiabetogenic doses of extracts of these glands.

The next experiment shows that diethylstilbestrol has a protective action in adrenalectomized moderate diabetic rats, with forced feeding, after subtotal pancreatectomy.

Male, white partially pancreatectomized rats were used. After the onset of diabetes forced feeding was begun. After 5 weeks, when constant glycemia and glycosuria was established, adrenalectomy was performed.

One week later the animals were divided in two groups; the animals of the control group received 25 mg of lactose daily, subcutaneously, in 0.5 ml of 0.9 % NaCl solution; the animals of the second group were injected daily with the same dose of lactose in the saline solution and with 50 µg of diethylstilbestrol dipropionate in addition. The animals drank, instead of water, 0.9 % NaCl solution *ad libitum*.

Results.

In agreement with the results of Long *et al.* ([22]) in rats fed *ad libitum*, table XI shows nearly a 25 % fall in the glycemia in forced fed rats, 7 days after adrenalectomy. Between the 7th and the 21st day. 55 % (12 of 22) of the animals died as a result of adrenal insuficiency. The diabetic state increase in those rats surviving one month until the preoperative level is again reached. This increase of the glycemia is concurrent with the development of accesory adrenal tissue.

In the adrenalectomized, partially pancreatectomized forced-fed rats injected with diethylstilbestrol, a toxic action of the estrogen was shown: 72 % (17 of 24) died, while the mortality of the control animals was only 55 %.

Of the 7 surviving rats, the diabetes increased in 3, similarly to the controls; but in the other 4 rats glycemia fell to normal levels and remained there until the end of the experiment, two and a half months later (chart 9).

Autopsy findings in the diethylstilbestrol group consisted of enlargement of the pituitary gland and marked atrophy of the testes. The accessory adrenals, which were visible to the naked eye in 6 out of 8 of the control rats, were much more marked in all the estrogen-treated animals, and were both uni and bilateral (table XII).

These results can be assumed to be due to the well-known hypertrophic action of the estrogens on the adrenal glands ([27]).

The microscopic study shows, in the diethylstilbestrol treated normoglycemic rats, hyperplasia of the insular tissue, with hypertrophy in some cases; newly formed islets occupied part of a pancreatic lobule. The diabetic rats showed atrophy of the islets with degranulated and vacuolized beta cells.

TABLE XI

Influence of adrenalectomy on the glycemia (mg per 100 ml) of forced-fed male diabetic rats.

Number of animals	GLYCEMIA	
	Before operation	1 week later
46	199	157

TABLE XII

Pituitary, accessory adrenals and testis weight (mg % body weight) of adrenalectomized and 95 % pancreatectomized male diabetic rats under forced feeding. Average of 5 injected with diethylstilbestrol and 7 controls (treated during two and a half months).

	Diethylstilbestrol 50 μg/day	Lactose
Hypophysis	15.5	3.7
Accesory adrenals	24.2	8.5
Testes	144	1066
Final body weight g	239	265

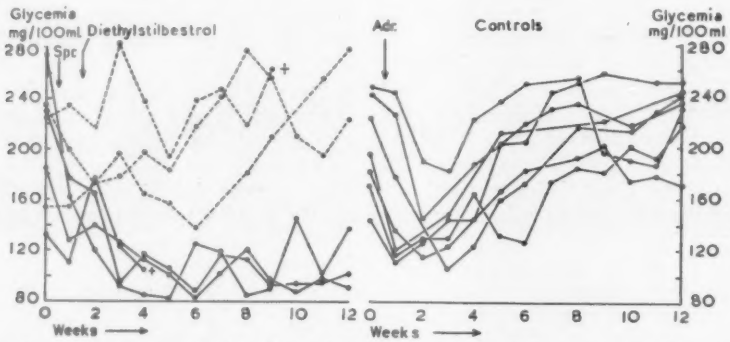

CHART 9. — *Variation of the glycemia of adrenalectomized and partially pancreatectomized male rats, under forced feeding, injected daily with 50 μg of diethylstilbestrol and controls (Rodríguez, 1950).*

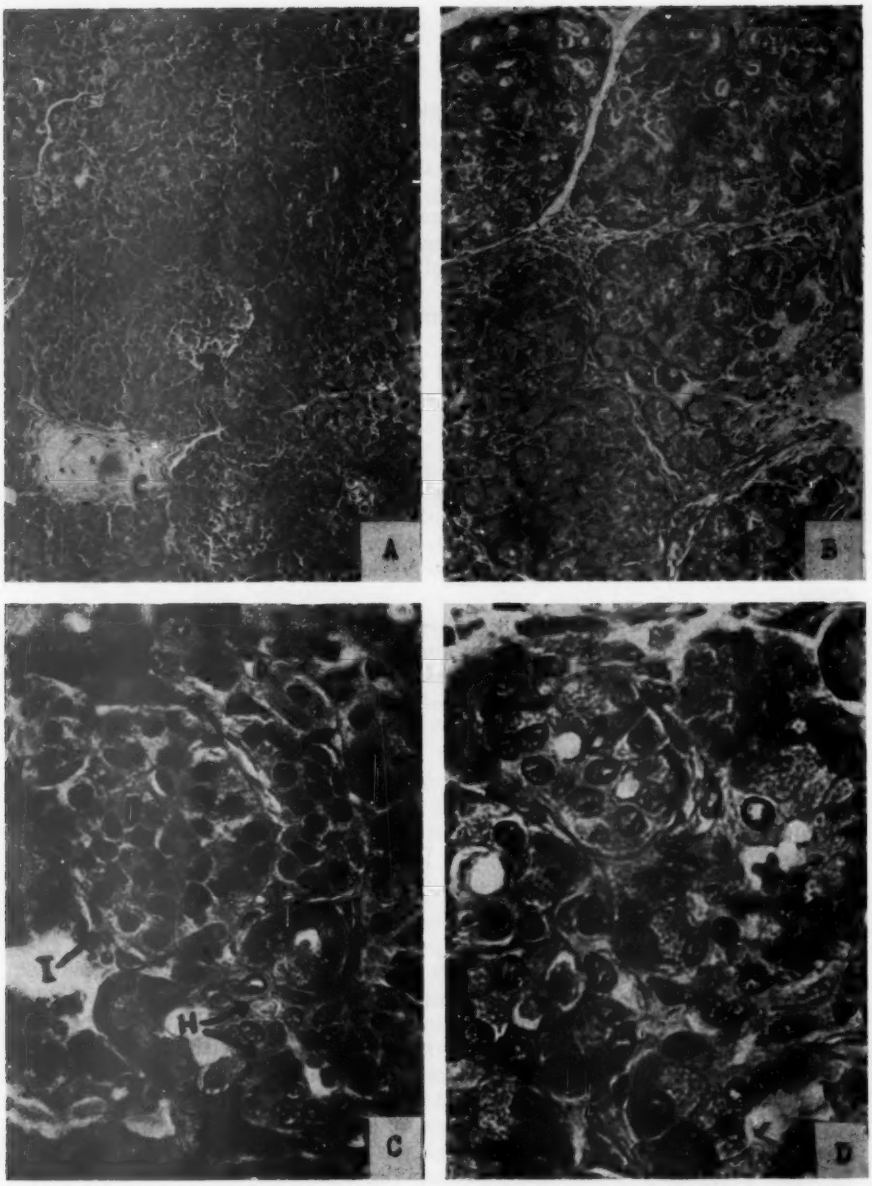

FIG. 3. — *A) normal cat pancreas. I, islet of Langerhans; C, sensory corpuscle (60 ×, hematoxylin-eosin). B) cut pancreas with local injection of estradiol. Diffuse hyperplasia of centroacinous cells, especially in H (120 ×, hematoxylin-eosin). C) section showing: 1, islet of Langerhans; H, hyperplasia of centroacinous cells, in the cytoplasm there are granules like beta granules (300 ×, Gomori's chromic hematoxylin). D) newly formed centroacinous cells with beta granules. The arrow shows compression of the acini by the centroacinous cells (540 ×, Gomori's chromic hematoxylin).*

The accesory adrenals, in the diethylstilbestrol injected rats, seemed to be round bodies, simple or lobulated, sorrounded by a condensation of capsule-like connective tissue. The cells appeared to be irregular shapes, without orientation, separated by many sinusoidal dilatated vessels. Cytologically, in the external zone there were cells of glomerular type, grouped in an irregular manner. The predominant type were the cells of the fascicular zone arranged in strands radiating from the central part and isolated, as were the cells of the external zone, by dilatated vessels. We were unable to see chromaffin cells, or cells from the reticular zone.

Therefore, these results seem to indicate that the protective action of the diethylstilbestrol is exerted through the adrenal glands.

3) *Direct action on pancreas.*

In this experiment, made in colaboration with Dr. A. F. Cardeza ([2]), normal rats, guinea pigs and cats were used. In the first experiment 6 rats of 150 g body weight, under ether anesthesia, were injected with 2 mg of estradiol benzoate, in the peripiloric region of pancreas. After 7, 15 and 30 days the animals were sacrified in groups of 2 for histologic study.

In the second experiment, 3 guinea pigs of 250 to 300 g body weight were injected in the same manner and with the same dose of estradiol. After 30 days they were examined histologically.

In the last experiment, 4 cats weighing between 1 and 2 kg, under ether anesthesia, were operated on; laparotomy was performed and, after removal of a small portion of pancreas for histological study, a pellet of 100 mg of estradiol was put into the mass of the pancreas. Two animals were sacrified after one month and the others after 2 and 6 months. Comparison was made between the control biopsies and the pancreas obtained in the autopsie. In all the cases the pancreas was fixed in Bouin-Hollande solution and stained with hematoxylin-eosin, Mallory-Azan and Gomori's chromic hematoxylin.

Results. — In the rats there was no change after 7 days of the injections. In animals injected for 15 days there was slight hyperplasia in the centroacinous cells, without any change in the number of islets. After 30 days the hyperplastic reaction was more marked and hypertrophied cell, similar to beta cells, were found in contact with centroacinous cells. The newly formed cells displaced the acini which showed signs of damage due to compression.

In the guinea pigs, after 15 days of estradiol implant, an increased number of islets was shown in comparison with the non injected controls. In some sections it was possible to see the continuity between acini and islets by means of the hyperplastic centroacinous cells.

The cat's pancreas, after one month of the implantation, showed a diffuse hyperplastic proliferation of centroacinous cells, with atrophy due to compression, of the exocrine tissue. These centroacinous cells showed granules in their cytoplasm, similar to those of the beta cells. After 2 months, the same changes were present.

Six months after the implantation very marked hyperplasia was shown in the centroacinous cells, some of which showed beta granules in their cytoplasm. The number of islets, in the zone near the pellet was considerably increased in comparison with sections made from biopsy tissue before the implantation (fig. 3).

In all the cases normal fasting blood sugar levels were found; the histology of adrenals and pituitary glands was normal in all the animals.

GENERAL CONSIDERATIONS

Sex difference.

A sex difference in relation to the onset and incidence of diabetes in rats of the same age has been further corroborated. The frequency was much higher in males than in females 6 months after operation (89 % and 27 % respectively). An alimentary factor as the exclusive or principal cause of the sex difference can be excluded because this difference persists in experiments in which pancreatectomized rats were pair-fed or forced-fed.

Gonadectomy.

In male rats, simultaneous castration reduces the incidence of diabetes caused by pancreatectomy. If castration is carried out after the onset of diabetes, the diabetes remains unchanged.

On the other hand, ovariectomy increases greatly the incidence of pancreatic diabetes. The protective action exerted by the hormone secreted by the ovary in physiological conditions is shown by grafting these organs in the kidney of ovariectomized-pancreatectomized rats; after 6 months a lower incidence of diabetes is shown in these animals, compared with ovariectomized-pancreatectomized controls (7).

Sex hormones.

In castrated rats after subtotal pancreatectomy, the daily injections of estrogens, for 6 months, had a marked protective action against the onset of diabetes. The following substances were used; estrone, estradiol, diethylstilbestrol, 1-methyl-bisdehydro-doisynolic acid, dienestrol, ethynil-estradiol and ethynil-testosterone. Cholesterol, although it had no estrogenic effect, also produced a slight but definite decrease in the incidente of diabetes.

This protective action in rats fed *ad libitum* is also observed in forced-feeding experiments. In this condition diethylstilbestrol has a marked protective action, preceded in some of the animals by a transitory stage, lasting for about one month, with an increase of the blood sugar level and glycosuria; this transitory stage is followed by the permanent protective action which takes place in all the diethylstilbestrol treated rats, with normal values in glycemia and glycosuria, in contrast with the increase of these values observed in non-injected controls. These preventive effects persist 6 months after the interruption of the treatment with estrogens.

Androgens, on the contrary, increased the incidence and accelerated the onset of diabetes. Substances with progestational activity such as progesterone, corticoids (desoxycorticosterone) and 17 β-ethyl-dihydro-

testosterone, had no effect on the course of diabetes in the castrated white rats.

After 95 % pancreatectomy, a compensatory hypertrophy of the insular system is observed histologically; such hypertrophy is similar to that produced in nearly all organs following partial removal of their mass. In the pancreas it was possible to see hypertrophy of the islets of Langerhans, transformation of some centroacinous cells to beta cells, with the granules being well preserved and stained normally, and some sclerosis due to peri-insular growth of connective tissue with penetration between the islet cells.

If the diabetic state does not develop, the compensatory hypertrophy persists even after one and a half year of the subtotal pancreatectomy, with moderate sclerosis. If diabetes appears, the hyperglycemia or another unknown factor produces an inhibition of the compensatory hypertrophy followed by insular atrophy and the appearance of degranulated and vacuolized beta cells with pycnotic nuclei. Marked sclerosis, peri- and intrainsular, is observed.

The gonads and sex hormones play an important part in this compensatory hypertrophy. The androgens interfere with it and for this reason increase the onset and incidence of diabetes.

The estrogens, on the contrary, produce hypertrophy and hyperplasia in the old islets and create new ones from the centroacinous cells. This is perhaps the most important cause of the marked protective action on the incidence of diabetes exerted by the ovarian hormones. This hypertrophy and hyperplasia persist even 6 months after the interruption of the estrogenic treatment.

This action is observed also in rats with moderate diabetes and future experiments will examine the possibility of ameliorating it.

Mechanism of the action of estrogens.

The effect of estrogens may be due to action on: a) pituitary gland; b) adrenal gland; c) direct stimulating action on the pancreatic islets and; d) peripheral metabolic action on the liver, muscle or other tissues. We have only studied the first three possibilities.

A) *Action on the hypophysis.* — From the fact that: 1) no significant changes are shown in the volume of the islets of Langerhans in hypophysectomized rats; 2) the initial increase of glycemia, due to diethylstilbestrol administration, took place in hypophysectomized-pancreatectomized forced-fed rats maintained with subdiabetogenic doses of pituitary extract and; 3) the injection of chorionic or serum gonadotrophin has no action on the glycemia or glycosuria of pancreatectomized rats with moderate diabetes, in forced-feeding experiments, we conclude that the action of estrogens in carbohydrate metabolism and diabetes, through the hypophysis, is not the only one and there are other mechanisms of action.

B) *Action on adrenals.* — The protective action of diethylstilbestrol in partially pancreatectomized rats was found to occur in andrenalectomized animals.

C) *Direct action on pancreas.* — Normal rats, guinea pigs and cats, with intrapancreatic implants of estrogens showed, in the beginning, a hyperplasia of the centroacinous cells and subsequently newly formed islets containing hypertrophied cells of the beta cell type. The fact that the formation of new islet tissue was more marked in the zone of estrogenic implantation, and also the normal structure of pituitary and adrenal glands, makes it seem reasonable to suppose that the action of the estrogens is, partially at least, a direct one upon pancreatic tissue.

Summarizing: In the rat a marked sex difference exists in relation to the incidence, onset and severity of diabetes after subtotal pancreatectomy: the male being more susceptible than the female. Male hormones increase the incidence of this type of diabetes and the female hormones on the contrary decrease it. The mechanism of the protective action of estrogens seems to be due to the hypertrophy and hyperplasia of the islets of Langerhans which they cause.

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SOME EFFECTS OF THE RAPID INFUSION OF LIQUID INTO THE CIRCULATORY SYSTEM

The circulatory wave

J. L. DUOMARCO AND C. E. GIAMBRUNO

*(Instituto de Patología *, Facultad de Medicina de Montevideo - Uruguay)*

DURING the course of some experiments performed by one of us with Opdyke and other colaborators ([5]) we were surprised by the fact that a rapide injection of saline into the right auricle produced an almost immediate elevation of arterial pressure. This rise was already evident in the second cycle that follows the beginning of the injection.

In order to explain such rapidity in the transportation of the liquid mass necessary to produce that rise of arterial pressure, we thought of the possibility that the right auricular distension, secondary to the infusion, could produce a left to right displacement of the interauricular septum; due to this displacement the left ventricle would receive a greater volume of blood with the corresponding rise of arterial pressure.

However the following facts are not in complete accordance with this hypothesis: 1) the pressure in the left auricule is usually greater than that in the right auricle, and the injection of liquid into the latter produces an immediate increase of pressure, more considerable in the left than in the right auricle; 2) the injection of liquid into the left auricle does not produce an immediate rise of pressure in the right auricle.

It was possible too, that the injection of saline in any sector of the circulatory system, could determine a wave of pressure-volume moving at a relatively great velocity along the circulatory system. This wave would be similar to the one formed in the arterial sector by every ventricular systole and would be the cause of the arterial pressure rise that appears rapidly after the injection into the vein. Consequently, two velocities, or more exactly, two kinds of velocity can be distinguished in every point of the circulatory system: the *hydraulic velocity* and the one that we shall call *velocity of the circulatory wave.*

1) The *hydraulic velocity* v can be expressed mathematically as

$$v = \frac{G}{S}$$

being G the cardiac output and S the total cross-section area considered.

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* Director: Prof. J. P. Migliaro.

Naturally, clinical methods used to measure *circulation time*, refer to this velocity but only as an average value along a definite segment of the circulatory system.

2) The *velocity of the circulatory wave* V can be represented by Moens' formula (4), or by the more significant one of Bramwell and Hill (2), deduced from the preceding one.

$$V = \frac{3.57}{\sqrt{A}}$$

being A the increase % of the vessel volume, produced by a pressure rise of 1 mm Hg, and V the velocity expressed in m/s. The study of the pulse wave velocity iniciated by Weitbrecht (7), refers really to the velocity of the circulatory wave in a limited vascular sector. The venous pulse, and in some special conditions the capillary pulse could extend this study to other sectors. The rapid infusion of liquid into a point of the circulatory system constitutes a very adequate method to study the *circulatory wave* (C.W.) and its velocity (C.W.V.), because it determines a perturbation sufficiently intense as to propagate along all the system.

Obviously, the determination of the (C.W.V.), requires a very simple measurement of time, and a measurement of the *distance* covered by the wave, which offers the same difficulties found in the determination of the actual velocity of blood flow. In both cases this difficulty can be suppressed if it is supposed that in all animals of the same kind and of the same size, the distance between two homologous points of the circulatory system is the same. On that basis, the simple measurement of the time (circulatory wave time - C.W.T.), gives a value inversely related to the C.W.V., and that can be taken as an index of it; such as *circulation time* is an index of the *actual circulatory velocity*. If the case requires it, the differences in longitude of the vascular sectors due to the size and weight of the animals can be easily corrected having in mind that the homologous distances change with the lenght or with the cubic root of the animal weight.

From a theoretical point of view, there is no objection to the idea that a rapid variation of contents in a point of the cardiovascular system can determine the formation of a wave which propagates along this system. The object of the experiments now described is to study some aspects of this phenomenon.

MATERIAL AND METHOD

In total 25 dogs were used, anaesthetized with chloralose in a dosis of 0.1 g/kg or with chloral-morphine solution in a dosis of 0.5 cm^3/kg. In a series of 12, whose weights varied between 8 and 22 kg, a canula was put into the femoral vein and a volume of saline (or blood of the same animal) that varied between 5 and 20 cm^3 was injected in less that 1 s. In several points of the circulatory system (right auricle, left auricle, pulmonary artery, femoral artery), and in not more that three for each animal, manometers recording local pressure were also connected. Pressure was recorded in a smoked paper at the velocity of 2 cm/s. Special care was taken in putting the different recording needles in the same generator of the cylinder. It was demonstrated by means of some control experiments that inertia and conduction time of manometers do not introduce a significant cause of error in the measurements of C.W.V.

In some additional experiments, saline or blood was injected directly into the arterial system. In other experiments, the total suppression of the central nervous system function was produced by the method of A. Ozorio de Almeida (6), that consists in connecting the animal's subaracnoid space with the water pipe (previous suboccipital puncture), in order to produce a spinal hipertension with total ischemia of the nervous system, during 10 to 15 minutes. The object of these experiments was to suppress the reactional vasomotor effect produced by the C.W. and to transform the vascular system in a virtual inert elastic system. With similar object, dogs with denervation of both carotids and section of both vagi were used.

Finally, in other experiments some control measurements were done of the actual circulation time, using sodium cyanide in a dosis of 0.5 to 1 mg per injection into the femoral vein, and registering the time of appearance of the respiratory and arterial pressure modifications produced by the cyanide. The fraction of time corresponding to the elaboration of the carotid reflex was discounted from the interval measured; this time was measured by the same method but with the cyanide injected into the carotid artery.

The circuit covered by the C.W. presents great variations of distensibility in its different segments and even in the same segment if considered at different moments. The greater variations are found in the cardiac cavities whose distensibility is very great during diastole and very small during systole, and besides it changes in every instant. On the other hand the cardiac valves intermitently interrupt the mentioned circuit. All this circumstances cause important variations of the C.W.V. in experiments performed in the same animal but at different moments of the cardiac cycle. For example, the C.W. that begins to pass from the ventricle to the arterial trunk in a certain venticular systole could have reached the corresponding auricle in any moment of the complete cardiac cycle, which ends with the closure of the auriculoventricular valves. This creates a margin of indetermination for each experiment more or less equivalent to the duration of one complete cardiac cycle. This cause of dispersion of the values obtained in experiments performed in the same animal tends to increase when the C.W. passes through both ventricles.

The arrival of the C.W. to the auricles is detected by a variation that can affect either the auricular systole or the auricular diastole; the arrival of the C.W. to the pulmonary or systemic arteries can be matter of discussion; we have taken as such moment the beginning of the first modified arterial wave.

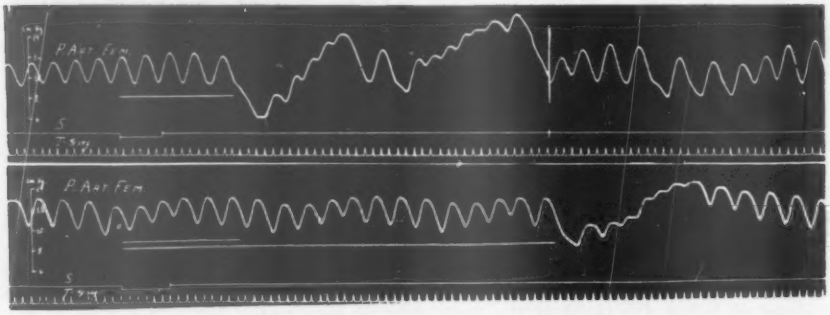


FIG. 1. — *Dog, 13 kg, anest.: chloral-morphine. (P. Art. Fem. = Femoral arterial pressure). A) Injection of 0.75 mg NaCN into carotid artery. Arterial pressure fall 2.7 s later. B) Injection of 0.75 mg NaCN into femoral vein. Arterial presure fall 9.2 s later.*

RESULTS AND DISCUSSION

Figures 1, 2 and 3 correspond to some experiments whose object was to establish some clear differences between the actual *circulatory velocity* and *circulation time* and what we have called C.W.V. and C.W.T.

In figure 1-A the injection of sodium cyanide into the carotid artery produces a fall of the arterial pressure, 2.7 s after the injection. If we suppose that the action of the cyanide is due to an excitation of the carotid

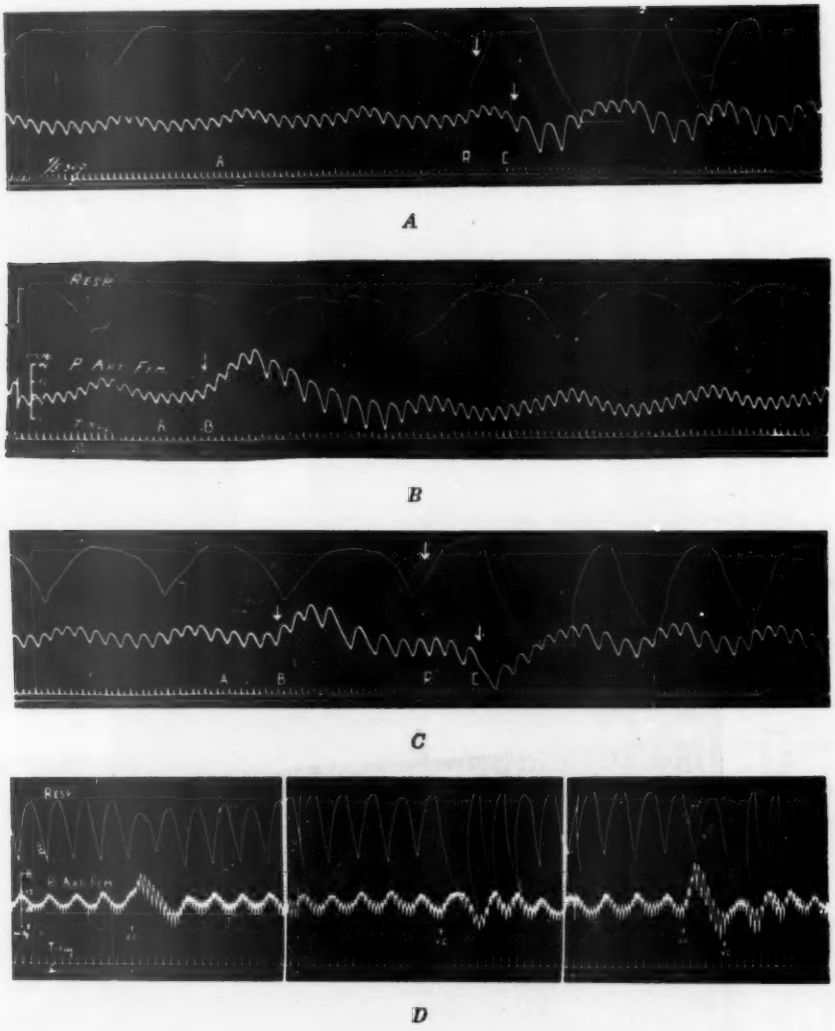


FIG. 2. — *Dog, 8 kg, anest.: chloral-morphine. (P. Art. Fem. = Femoral arterial pressure). A) Injection of 1 mg NaCN into femoral vein (A). Circulatory answer (C) 9 s after the injection. Respiratory answer (R) 7.8 s after the injection. B) Injection of 20 cm³ into femoral vein (A). Circulatory wave reaches femoral artery 1.7 s after injection (B). C) Injection of 20 cm³ saline with 1 mg NaCN into femoral vein (A). Successively: arrival of circulatory wave (B), respiratory (R) and circulatory (C) answers to cyanide. D) Three stages of the same experiment at a reduced velocity.*

sinus, we can admit that this interval is the latency time of the corresponding reflex.

In figure 1-B the cyanide injected into the femoral vein produces the circulatory answer 9.2 s after the injection; the femoral vein-carotid artery circulation time is thus 9.2 — 2.7 = 6.5 s.

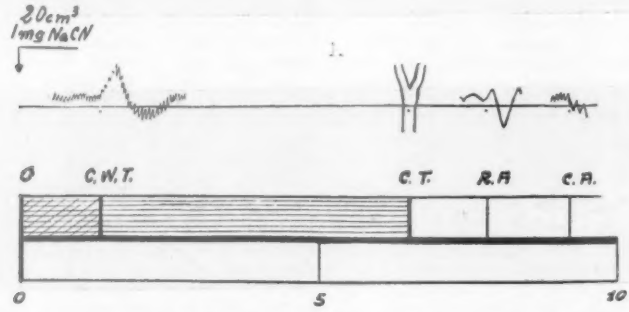
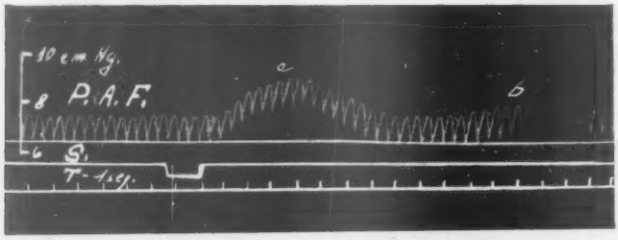
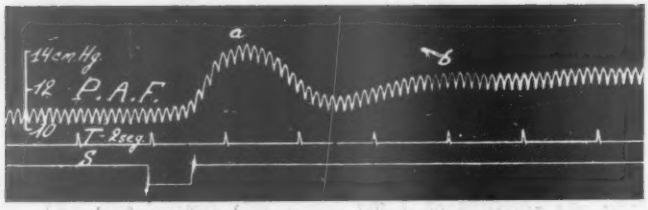


FIG. 3. — *Diagram summarizing experiments in figures* 1 *and* 2. *O: beginning of an injection of* 20 *cm³ of saline with* 1 *mg NaCN into the femoral vein. C.W.T.:* circulatory wave time *(femoral vein-femoral artery). C.T.:* Circulation time *(femoral vein-carotid sinus). R.A.: respiratory answers. C.A.: circulatory answer.*



A



B

FIG. 4. — *Dog,* 10 *kg, anest.: chloralose. Exclusion of central nervous system by means of spinal hypertension.* 20 *cm³ of saline injected into the femoral vein. It is observed an initial positive wave* a *followed by a positive secondary wave* b. *B) Dog,* 9 *kg, anest.: chloral-morphine. Denervation of both carotid sinus and section of both vagi. The* 20 *cm³ saline injection produces the same effect as in the preceding experiment. (P.A.F. = femoral arterial pressure).*

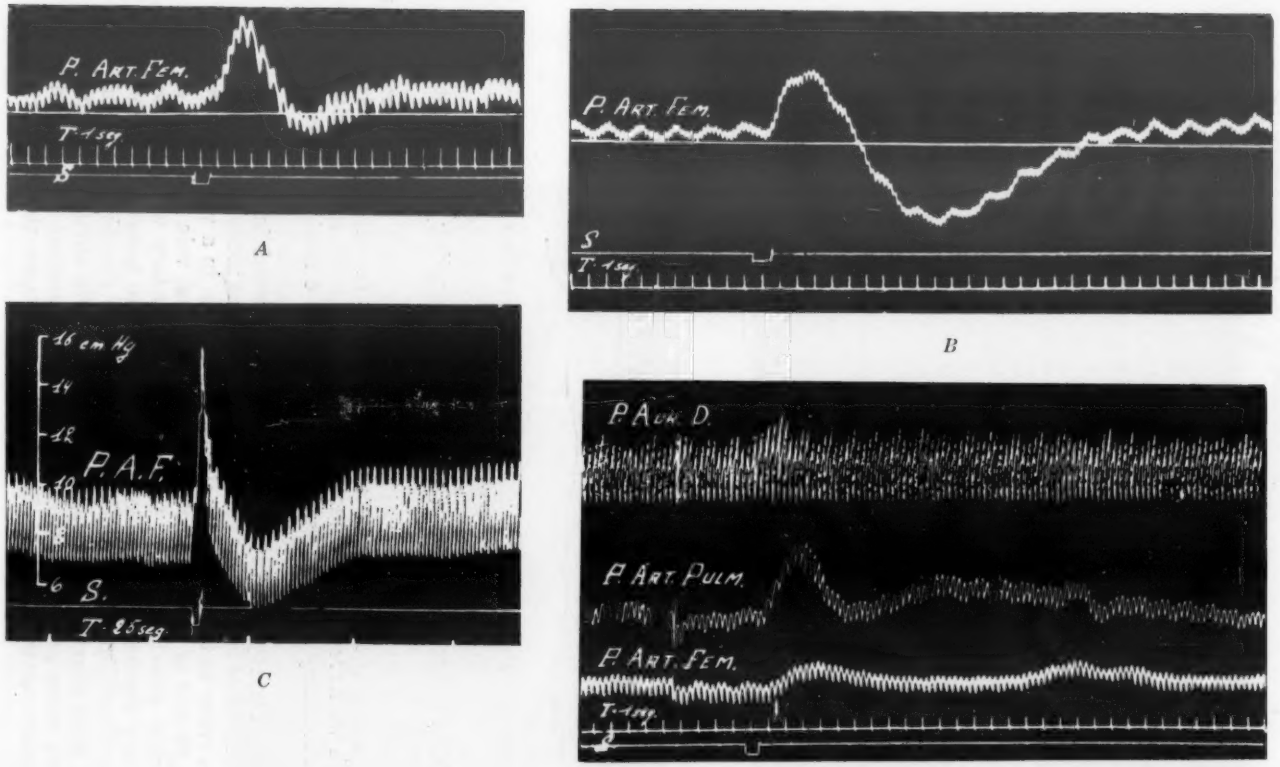


FIG. 5. — *A) Dog, 13 kg, anest.: chloral-morphine. 20 cm³ of saline injected into the femoral vein. Biphasic wave in the femoral artery. B) Dog, 9 kg, anest.: chloralose. Same experiment. C) Dog, 7 kg, anest.: dial. The 20 cm³ of saline injected into one of the femoral arteries produces a biphasic wave in the other femoral artery. It is remarkable the rapidity and sharpness of the positive portion. D) Dog, 20 kg, anest.: cloralose. Opened thorax, artificial respiration. The biphasic wave prolongs on passing successively through the right auricle, the pulmonary artery and the femoral artery. P. ART. Fem. = Femoral arterial pressure; P. Aur. D. = right auricular pressure; P. Art. Pulm. = pulmonary arterial pressure.*

In figure 2-A we observe that the circulatory answer to the cyanide injection has a delay of 9 s, but the respiratory answer iniciated by a greater rapidity of expiration occurs 1.2 s before the preceding one.

In figure 2-B the injection of 20 cm^3 of saline into the femoral vein produces a C.W. that reaches the femoral artery only 1.7 s after the injection.

Figure 2-C shows in the same experiment the C.W. and the actual circulation time. The injection of 20 cm^3 of saline containing 1 mg of sodium cyanide produces a C.W. that reaches the arterial system 1.7 s after the injection; on the other hand, the arrival of the cyanide to the carotid sinus produces the respiratory answer 6.4 s and the circulatory one 8.3 s after the injection.

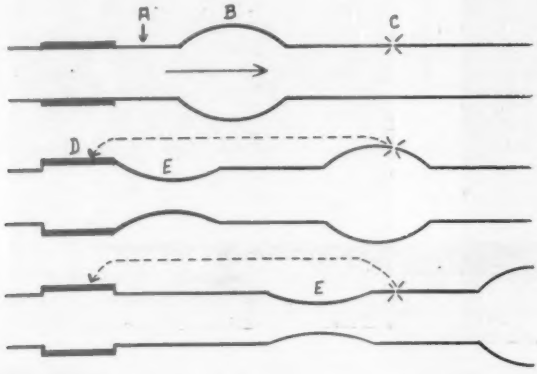
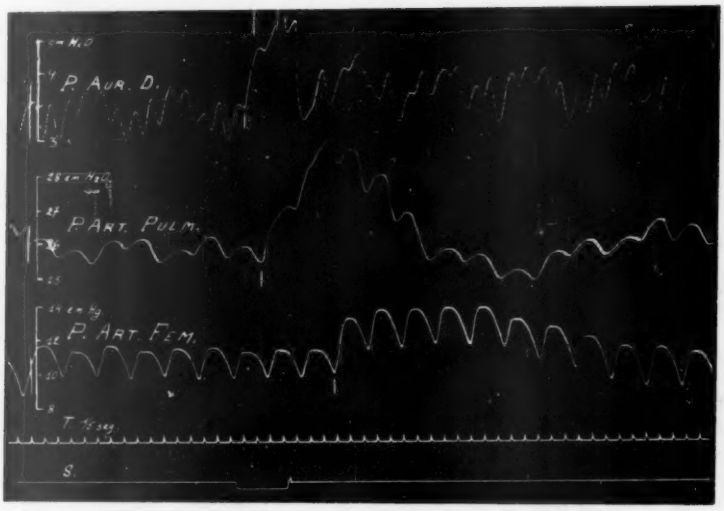


FIG. 6. — *Diagram showing the formation of the biphasic wave (see text).*

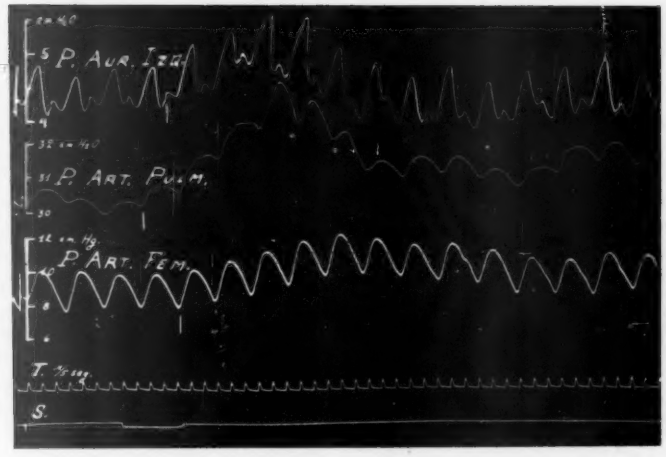
Figure 2-D represents the three stages of the preceding experiment at a reduced velocity.

The diagram in figure 3 summarizes the results of the first series of experiments. Since the velocity of the C.W. in the arterial system is relatively high we have supposed that the C.W. reaches the carotid sinus and the femoral artery simultaneously; the error thus commited is insignificant.

Figure 4-A corresponds to an experiment performed in an animal which was deprived of all nervous central activity by the method indicated previously, in order to transform its circulatory system in a virtual inert system. The 20 cm^3 of saline injected into the femoral vein produced a positive principal arterial wave *a*, followed by a secondary wave *b*, also positive but very flattened and prolonged. It is easy to imagine that the secondary wave corresponds to the reentrance in the arterial system of the principal wave considerably damped by one complete circulatory tour. This secondary wave probably extends all over the vascular system and slowly disappears as the injected liquid filters throught the capillary walls.



A



B

FIG. 7. — *Dogs 4 and 5 of figure 8, anest.: chloralose, opened thorax, artificial respiration; 20 cm³ of saline injected into femoral vein. A) The circulatory wave passes successively through the right auricle, the pulmonary artery and the femoral artery. B) The circulatory wave passes successively through the pulmonary artery, the left auricle and the femoral artery. P. Aur. D = right auricular pressure; P. Aur. izq. = left auricular pressure; P. Art. Pul. = pulmonary arterial pressure; P. Art. Fem. = femoral arterial pressure.*

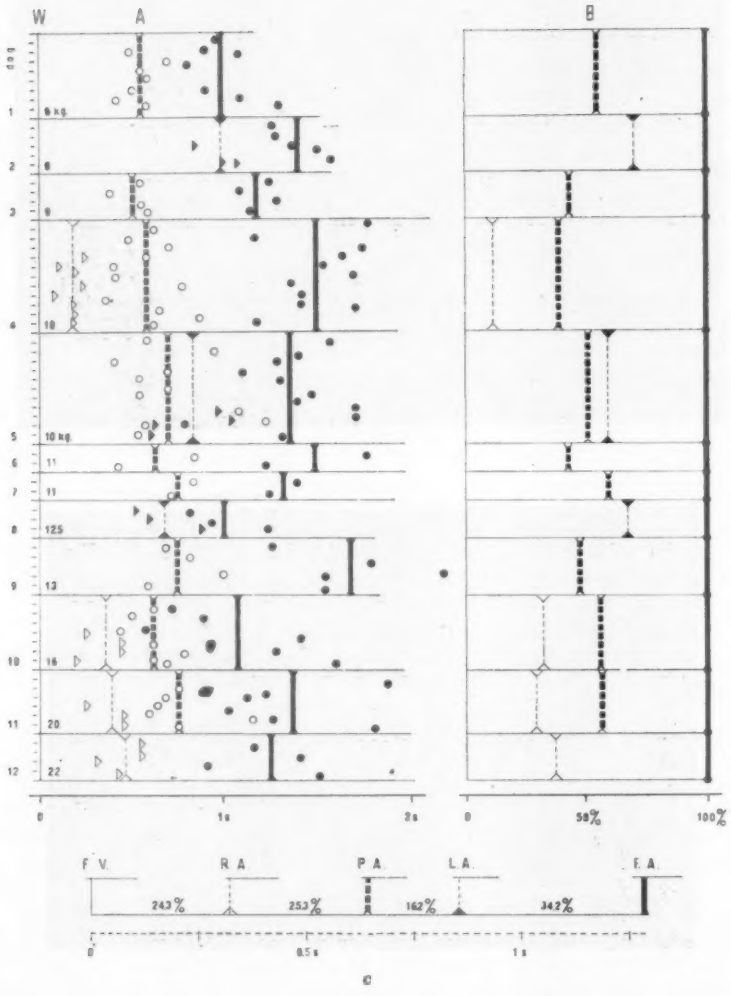


FIG. 8. — *A) Results obtained in 68 experiments performed in a series of 12 dogs ordered according to their weight. The left vertical line marks the beginning of injection of 20 cm³ of saline into the femoral vein. White triangles mark arrival of C.W. to right auricle, white circles to pulmonary artery, black triangles to left auricle, black circles to femoral artery. The corresponding vertical lines represent partial averages. B) The same partial averages supposing the femoral vein-femoral artery time equal to 100. C) Total averages of the 68 experiments.*

Figure 4-B is very similar to the preceding one and corresponds to an experiment performed in a dog with section of both vagi and denervation of both carotid sinus.

In any other of the figures that correspond to experiments of rapid injection of liquid into animals with intact nervous system, we can observe that the principal wave is immediately followed by a secondary negative wave. This wave has a surface comparable to that of the first one and can be followed by other waves, alternatively positive and negative but of a much smaller amplitude.

Figure 5-A-B-C shows different aspects of this biphasic wave as it passes through the arterial system. Figure 5-D shows the prolongation of the biphasic wave as it passes successively through the right auricle, the pulmonary artery and the femoral artery.

The interpretation of the biphasic wave is not difficult if we have in mind the present knowledge about the functions of the carotid sinus ([3]). A positive wave *B* generated by a rapid infusion of liquid into a point *A* of the circulatory system moves towards the pressoreceptors *C* (fig. 6); on reaching them, a relaxation of the wall, of reflex origin, is produced in sector *D;* consequently a negative wave *E* is generated that moves in the same direction that wave *B*. It is understood that if both waves are sufficiently long, they partially unite and create the biphasic wave observed in the records.

The possibility that the negative wave might be due to a decrease of blood viscosity, because of dilution with the injected liquid, is easily discarded if we have in mind that the C.W. does not substantially change when instead of saline, blood of the same animal, extracted a moment before, is injected.

Figure 7 corresponds to some experiments performed with great velocity of the cylinder, in order to measure the moments in which the C.W. reached the different sectors of the circulatory system. It can be appreciated that the wave runs successively from the right auricle to the pulmonary artery, the left auricle, and the aortic system, and that consequently a direct passage through the interauricular septum does not exist.

Figure 8 summarizes the data obtained in 68 experiments performed in a series of 12 dogs ordered according to their weight. Vertical line *O* represents the beginning of the injection into the femoral vein and the different figures the moment in which the circulatory wave reaches the four selected points of the circulatory system. The partial averages are represented by corresponding vertical lines. It is observed that even for the same animal, the dispersion of values corresponding to successive determinations is considerable. We have referred before to the cause of this dispersion of values that naturally determines fairly great variations in the partial averages and in the percentages of those averages supposing the femoral vein-femoral artery C.W.T. equal to 100.

The experimental data show that the C.W. that starts in the femoral vein arrives to the femoral artery in an average time of 1.319 s with a standard deviation of ± 0.251 s; that almost half of that time (0.625 s ± 0.167 s) is used in reaching the pulmonary artery; almost one quarter

(0.298 ± 0.083 s) in reaching the right auricle, and almost two thirds (0.832 ± 0.277 s) in reaching the left auricle.

A rapid statistical analysis of these figures does not object the hypothesis that the principal cause of variancy, in the same animal, is the indetermination introduced in the C.W. circuit by the cardiac activity.

Figure 8 does not allow us to appreciate any clear relationship between C.W.V. and animal weight.

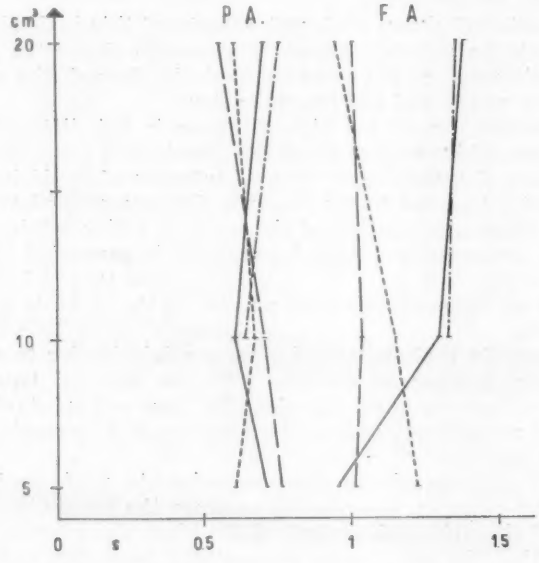


FIG. 9. — *Arrival of C.W. to pulmonary artery and femoral artery when the volume injected varies between 5 and 20 cm³.*

Figure 9 summarizes the averages of several experiments performed in 4 animals, in order to measure the moments in which the C.W. reached the femoral artery, when the volume of saline injected varied between 5 and 20 cm³. As it could be expected, the C.W.V. is independent of the injected volume.

The study of the interval of time necessary to cross the systemic capillary sector has some special difficulties related to the problem of the sense in which the wave is propagated. It is easily understood that after a rapid injection into any point of the circulatory system, a belly is formed that expands in both senses and that consequently generates two waves: one of these waves, the only one considered up to this moment, moves in the sense of blood flow: it is the progressive wave; the other one moves in the opposite direction; it is the retrogressive wave. If saline is injected into the femoral vein, the retrogressive wave is partially damped in the capillary system and partially reflected in the venous

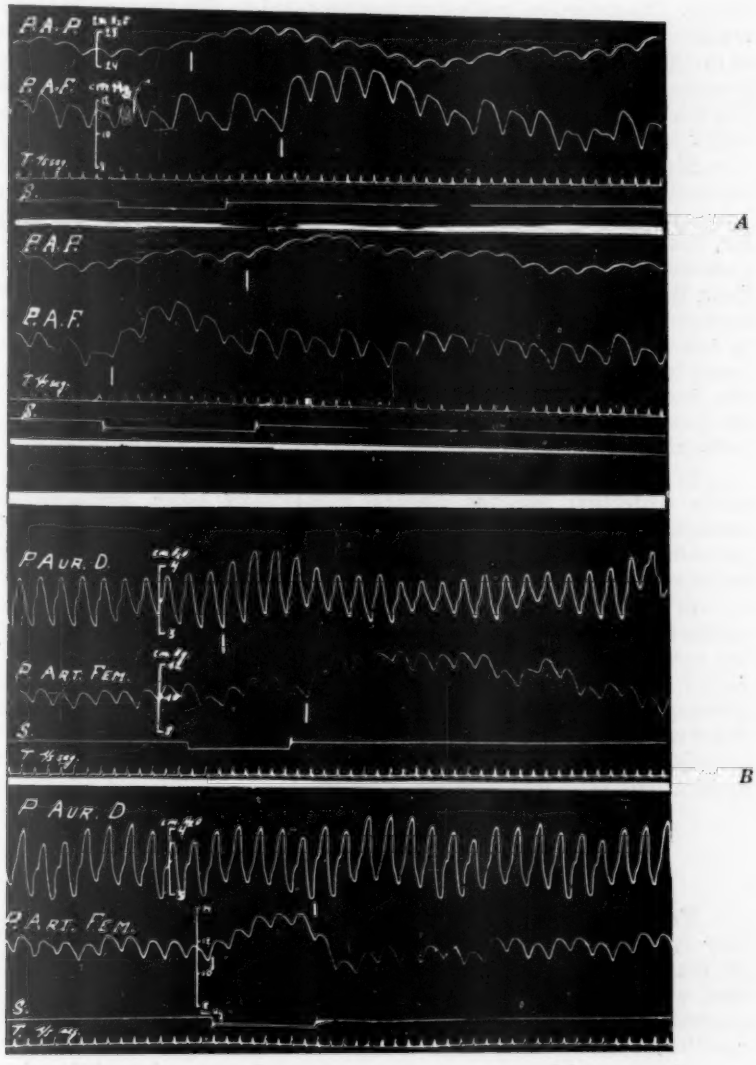


FIG. 10. — *Dogs 9 and 12 of figure 8; anest.: chloralose, opened thorax, artificial respiration. A) Rapid injection of saline into femoral vein (above) and into femoral artery (below). Records of pulmonary (P.A.P.) and systemic arterial pressures (P.A.F.). B) The same injection with records of right auricular (P. Aur. Der.) and femoral arterial pressure (P. Art. F) (see text).*

valves. The reflected portion is incorporated to the progressive wave. The reentrance of the progresive wave into the venous system after its passage through the arterial system and the capillaries is not very nitid because it has been damped by the very distensible capillary sector; on the other hand when the wave reaches the arterial system, it finds the latter submitted to the secondary oscillations of the injection.

Figure 10 illustrates some attempts to measure the C.W.V. in the systemic capillary sector having in mind that in a vascular system the velocities of the retrogressive and progressive waves must be very similar, but affected by the same cause of dispersion mentioned before. In figure 10-A, the wave produced by an injection into the femoral vein goes from the pulmonary artery to the femoral artery in 1.4 s; if the liquid is injected into the femoral artery, the wave reaches the pulmonary artery in two seconds. The difference of 0.6 s between the two intervals is too small to decide if in the last case the wave produced by the injection into the femoral artery reached the pulmonary artery by retrogressive way, or instead, by progressive way with a previous passage through the systemic capillaries.

In figure 10-B the right auricle-femoral artery time in the direct sense (injection into the femoral vein) is of 1.5 s, while the femoral artery-right auricle time (injection into the femoral artery) is of 1.6 s; we can not deduce from these figures whether the transmission is retrogressive, or direct through the capillary system.

It is evident that the problem of the C.W.V. through the systemic capillaries can not be solved with these elements. Bare indications of the value of the C.W.T. corresponding to a complete circulatory tour are found in figure 6; it is observed that the time elapsed between the beginning of the initial and secondary waves is of 4.2 s in the dog with denervation of both carotid sinus, section of both vagi and normal arterial pressure; and of 7 s in the dog with exclusion of central nervous system and very low arterial pressure.

Summary and conclusions

From a certain point of view, it is important to consider the circulatory system as a continuous elastic tube. Obviously, its distensibility and its distension varies with the different sectors (veins, arteries, capillaries, cardiac cavities) and with the different moments (contraction or relaxation of the vascular territories, systole or diastole of the cardiac cavities) for each sector of that tube.

The rapid injection of saline or blood into a point of the circulatory system determines the appearance of a wave (circulatory wave) that moves along this system with a velocity much greater than, and independent of, the circulatory velocity. The pulse wave velocity is a particular case of the circulatory wave velocity in the systemic arterial sector.

The circulatory wave is formed by a positive principal wave followed by a secondary negative one. The origin of this secondary wave is

discussed by means of some experiments with functional suppression of the nervous system.

The time of passage of the circulatory wave in the different sectors of the cardiovascular system (between the femoral vein, the right auricle, the pulmonary artery, the left auricle and the femoral artery) was determined in a series of 12 dogs. The values obtained are fairly dispersed due to the variations produced by the elastic changes of the cardiac walls and the closing of the valves and are also independent of the volume of liquid injected and of the animal weight; the femoral vein-femoral artery time is, in average, of 1.3 s.

It was demonstrated in some control experiments that the actual circulatory time corresponding to this same sector and measured by the method of the sodium cyanide varied around 7 s.

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RENIN BINDING BY THE TISSUES

J. R. E. SUÁREZ AND J. C. FASCIOLO

(Instituto de Fisiología, Universidad Nacional de Tucumán. Córdoba 451, *Tucumán).*

USING the most sensitive methods [9] minute amounts of renin could be detected in the blood of normal individuals or animals. However, no significant differences were found between normal and hypertensive subjects [21, 3] with the exception of the early stages of experimental nephrogenic hypertension [17, 20, 12]. In consequence, the present evidence appears to be against the rôle of renin as the "chemical mediator" in renal hypertension.

However, there remain two possibilities. Hypertensin may be formed within the kidney and in this form secreted. The recent work of Skeggs, Kahn and Shumway [19] does not seem to support that theory. They estimated hypertensin in normal and hypertensive dogs by dialyzing their blood with an artificial kidney. Here again differences were not significant excepting for the early stages of experimental hypertension, in which the blood was found to contain greater amounts of hypertensin *

Gregory *et al.* [11] were able to detect hypertensin in ultrafiltrates of citrated blood plasma of normal human beings or dogs during the pressure rise due to intravenous injection of hypertensin. No such increase in pressor substances could be demonstrated in ultrafiltrates of the blood of patients with chronic essential hypertension or in that of dogs with chronic experimental nephrogenic hypertension. They concluded that these results support the concept that hypertensin is not the effective pressor substance in experimental hypertension in dogs.

The second possibility is that renin may be absorbed in the arteriolar wall, and develop its action forming *in situ* small amounts of hypertensin. Houssay, Braun-Menéndez and Dexter [13] and Trautman *et al.* [22] perfusing isolated organs with blood or saline solution containing renin, were not able to show significant decrease in the renin of the perfused

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* The hypertensin found in the blood might be formed by the action of renin contained in blood.

liquid. On the other hand, Introzzi *et al.* [15] state that in traumatic shock, where the renin content of blood is greatly increased, renin is found in extracts of liver and notably in those of heart muscle. These conflicting results led us to reinvestigate the problem.

Since there are differences among renin from various species [7, 1] it seemed safer to work with homologous renin. This can be conveniently accomplished in shock experiments. In this condition it has been shown that renin content of blood is greatly increased [14, 4, 5, 6, etc.].

Methods

Experiments were performed on 9 dogs weighing 4 to 25 kg, anesthetized by intravenous injection of chloralose or nembutal. Biopsies were taken from liver, spleen and muscles of the abdominal wall, through a midline laparotomy. Shock was then induced in 8 of the dogs: in five by repeated hemorrhage; in one by trauma, according to the method of Best and Solandt [2]; anesthetic shock in other dog and surgical shock in the remainder. In all experiments the blood pressure dropped to 50 mm Hg or below. After a period of 15 minutes to 2 hours under such conditions, new biopsies from liver, spleen and muscle, and others from heart muscle (left ventricle) and renal cortex were taken, the last one as a test of complete hypertensinase destruction.

One dog was injected intravenously with 400 units of pig renin, which caused a pressure rise of 60 mm Hg. Twenty minutes later, with the pressure slowly falling to the previous level, biopsies were taken from liver, spleen and heart muscle.

The renin content of biopsies was estimated by the method of Fasciolo and Taquini [10]. In some experiments greater amounts of tissue extracts were employed than those used for the kidney by these authors, i.e. 2 or 3 mg of fresh tissue added to the hypertensinogen solution.

In addition, in a few experiments renin was estimated by a modification of the previous method, proposed by Fasciolo and Suárez [8]. Here the hypertensin formed is estimated by its pressor action on the dog. In that case, 12.5 mg of tissue were added to hypertensinogen and incubated.

Results

As can be seen in figures 1 and 2, in no case did liver, spleen, skeletal or heart muscles show consistent vasoconstrictor or pressor activity. This was true with the samples taken before and after the induction of shock. On the other hand, samples of renal cortex provoked, as expected, a marked vasoconstriction in the toad's hindlegs and a clear rise of pressure (about 30 mm Hg) in the dog.

Discussion

It is a well known fact that during shock, renin concentration in the blood increases [14, 4, 5, 6].

Introzzi *et al.* [15], using the method of Fasciolo and Taquini [10] stated that renin can be detected in heart muscle and liver of dogs after traumatic shock. They found, in the great majority of cases, a vasoconstrictor action with extracts of both tissues (greater with heart muscle). In one case, the vasoconstrictor effect reached a degree comparable with that found in the renal cortex of the same animal.

These authors characterized the constrictor action as due to renin bound by tissues in the special conditions derived from traumatic shock.

Trautman, Davis and Findley ([22]) made perfusions of isolated rabbit heart with Ringer solution containing renin. They found (with less sensitive methods), that renin solution provoked similar pressor responses in a cat before as well as after its perfusion through the heart. This suggested that renin had not been destroyed nor remained fixed in the heart in detectable amounts. Accordingly, saline extracts of perfused hearts showed no pressor action attributable to renin.

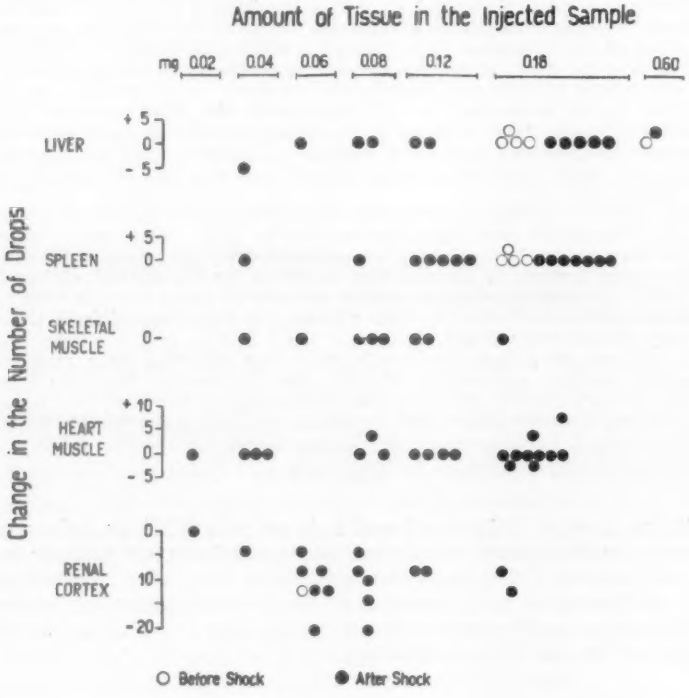

FIG. 1. — *Estimation of renin in the tissues. Hypertensin formed after incubation was tested by its vasoconstrictor action on the Läwen-Trendelenburg preparation. Figures on top correspond to the different amounts of incubated tissue contained in the final sample to be injected. The latter was always made up to 1 ml with Hülse's solution. Symbols represent the result of a single injection. Renal cortex shows a lack of correlation between tested amount of renin and vasoconstrictor activity. This is only apparent, because most of points correspond to different kidneys with uneven renin content. When a sample of renal cortex showed poor action it was tested in greater amount, always giving greater response. Tests with 0.5 ml standard solution of hypertensin usually gave a decrease of 8-12 drops.*

In our experiments, extracts from heart muscle, skeletal muscle, liver and spleen, treated as described above, showed no vasoconstrictor action before or after the shock or the injection of renin.

That is to say, in neither of these tissues could significant amounts of renin be characterized. On the other hand, the vasoconstrictor and pressor activity displayed by the renal cortex, shows that renin when present is easily detected in tissues.

Acording to Jiménez Díaz *et al.* (16), the vascular wall of any organ other than kidney, has an enzyme capable of acting upon hypertensinogen and give hypertensin. It is to be noted that none of the organs tested in our experiments, except kidney, showed renin-like activity, in spite of being well vascularized structures.

Houssay, Braun-Menéndez and Dexter (13) studied renin disappearance from blood. According to their results, renin should be fixed or destroyed by the tissues. Kidney should have a predominant, though not essential rôle, since renin disappears from the blood of bilateral nephrectomized dogs. The work of Leloir *et al.* (18) suggests that liver plays some rôle, but most of the destruction would be done by the remaining tissues. Our results, showing that tissues do not bind renin in detectable amounts, suggest that in some way they must destroy it.

FIG. 2. — *Estimation of renin in the tissues. Hypertensin formed after incubation was tested by its pressor action in the dog. Arrows mark the time of the intravenous injections. M = Skeletal muscle; C = Heart muscle; B = Spleen; R = Renal Cortex; O = Blank without tissue. All biopsies were taken after shock. The amount of incubated tissue contained in the samples to be injected were: renal cortex, 50 mg; remainder tissues, 100 mg each.*

SUMMARY

The presence of renin in heart muscle, liver, spleen and skeletal muscle was investigated in one dog injected with 400 units of pig renin and 8 other dogs in shock of several types.

Saline extracts of these tissues were incubated in the presence of dog hypertensinogen. Tests of the vasoconstrictor activity were performed in a toad's Läwen-Trendelenburg preparation, and of pressor action on dog.

Renin could not be detected before or after shock or renin injection in those tissues.

These results point against the possibility of renin being fixed by vascular tissues and there exerting its action by local liberation of small amounts of hypertensin.

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THE INACTIVATION BY OXYGEN OF THE ERITHROPOIETIC EFFECT OF PLASMA OF RABBITS RENDERED ANEMIC BY BLEEDING

B. GUNTHER, G. HODGSON, J. TOHÉ AND O. QUAPPE

(Institute of Physiology, University of Concepción, Chile)

THE EXISTENCE of an erithropoietic factor in the blood of anemic animals, first described by Carnot and Deflandre (2) in 1906, and again by Förster and Kiss (3) in 1925, has been denied (4). However, in recent years, erithropoietic activity has been found, not only in the blood of rabbits made anemic by bleeding (7), but also in that of rabbits and rats maintained in low pressure chambers (1, 9), as well as in that of patients with chronic heart failure (1), and in the blood of new born babies (6). This blood loses its erithropoietic effect when submitted to the action of pure oxygen (6).

The purpose of this paper is to describe experiments which demonstrate that oxygen also diminishes the erithropoietic activiy of plasma of rabbits rendered anemic by repeated bleedings.

MATERIAL AND METHODS

Ninetyone rabbits of both sexes were used. In a first series of experiments, the plasma obtained from rabbits rendered anemic by repeated bleedings was maintained up to the moment of injection in the receptor rabbits, free from contact with the air by a protective layer of liquid petrolatum. In a second series of experiments, the plasma obtained from the anemic rabbits was submitted to the action of a continuous current of oxygen, bubbling through it for 20 minutes, and then injected into the receptor animals.

As a control experiment, plasma obtained from normal rabbits was injected intravenously into another group of rabbits.

The receptor rabbits in all three sets of experiments, were bled once, previous to the injection of plasma. The volume of blood extracted was 10 ml per kg of body weight.

In the receptor rabbits receiving plasma of anemic animals maintained free from contact with the air, and in the control group, hemoglobin determinations, red cell and reticulocyte counts were made on two occasions before the injection, for 11 days after, and then again on the fifteenth day following the injection.

In the receptor group receiving plasma of anemic rabbits, submitted to the action of oxygen, only reticulocyte counts were made on two occasions before injection, and for nine days after. This was done, as it was seen that the only effect of one injection of anemic rabbits plasma maintained free from contact with the

air, on the peripheral blood picture of the injected animals, was an increase in the percentage of reticulocytes. This also was the only effect described by Krumdieck (4).

In all experiments, hemoglobin was determined as oxyhemoglobin in a Leitz photoelectric colorimeter; red cell counts were made in a Thoma chamber, 10 large squares being counted for each determination. Reticulocytes were stained with brilliant cresyl blue, and the percentage of reticulocytes in a thousand blood cells was established.

For the experiments described above the rabbits were grouped in the following manner:

Group A: Donor rabbits. — In all, 36 rabbits of both sexes, average weight 1 541 g were used. These animals were bled every other day by cardiac puncture on 5 occasions. The first four times, a volume of blood equal to 10 ml per kg of body weight was extracted. The volume of blood extracted on the fifth bleeding equaled 15 ml per kg of body weight. The plasma for injection was obtained from this blood. The plasma of 16 of the donor rabbits (group A_1, table I), was maintained under liquid pertrolatum, while that of the other 20 donors (group A_2, table I) was submitted to the action of oxygen before being injected.

TABLE I

Mean values and standard errors of the mean (SE) for hemoglobin (g per 100 ml) and the percentage of reticulocytes, of the donor rabbits before the first and fifth extractions of blood.

	1st control	2nd control
	Group A_1 (16 rabbits)	
Reticulocytes (%)	1.1 ± 0.14	16.4 ± 1.07
Hemoglobin (g per 100 ml)	9.0 ± 0.38	5.7 ± 0.41
	Group A_2 (20 rabbits)	
Reticulocytes (%)	1.6 ± 0.17	20.2 ± 0.85
Hemoglobin (g per 100 ml)	7.72 ± 0.2	5.42 ± 0.17

In this group of donor rabbits, hemoglobin determinations and reticulocyte counts were made before the first and fifth bleeding (see table I).

Group B_1: Receptor rabbits. — Seventeen rabbits of both sexes (average weight 1 542 g) were used. This group received plasma intravenously (10 ml per kg of body weight) obtained from anemic rabbits and maintained free from contact with the air up to the moment of injection. In the rest of this paper this group shall be referred to as group B_1.

Group B_2: Receptor rabbits. — Twenty rabbits of both sexes (average weight 1 744 g) were used. This group received plasma intravenously (10 ml por kg of body weight) of anemic rabbits, which was submitted to the action of oxygen,

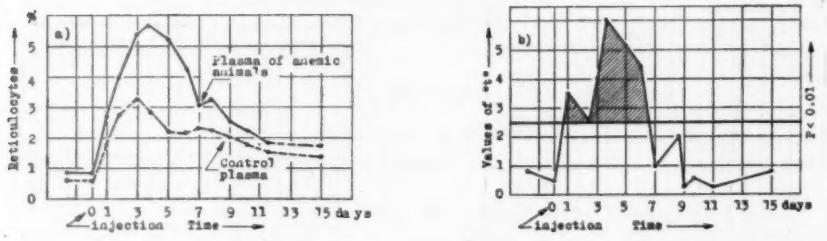

FIG. 1. — *a) Mean values for the percentage of reticulocytes of the control group injected with plasma of normal rabbits (discontinuous line — — —) and of the group injected with plasma of anemic rabbits, maintained free from contact with the air (continuous line ————). b) Values of the "t" test of significance (Fisher), for the estimation of the statistical significance of the difference between two means. It was considered that the difference between two means was significant when: $t > 2.5$, $P < 0.01$.*

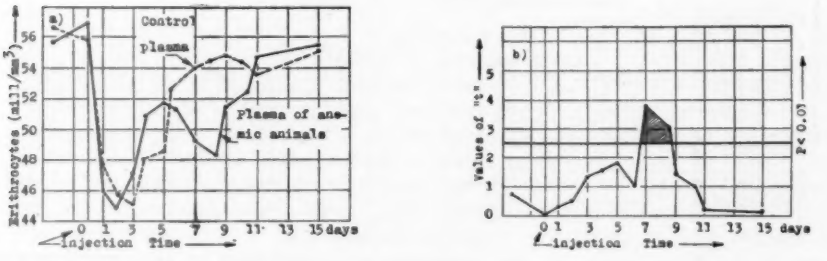

FIG. 2. — *a) Mean values for erithrocytes (millions per mm³) of the control group (discontinuous line — — —) and the group injected with plasma of anemic animals maintained free from contact with air (continuous line ————). b) Values of "t" for the difference between the means.*

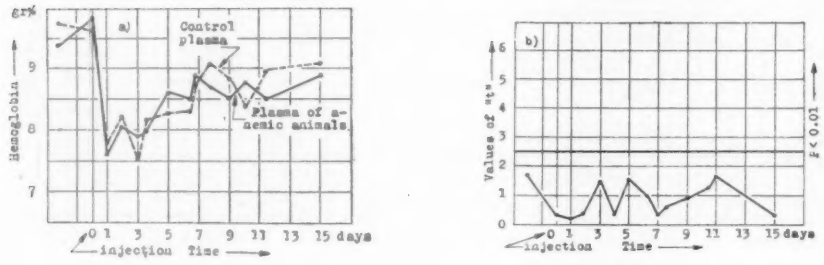

FIG. 3. — *a) Mean values for hemoglobin (g per 100 ml) of the control group (discontinuous line — — —) and the group injected with plasma of anemic rabbits maintained free from contact with the air (continuous line ————). b) Values of "t" for the difference between the means.*

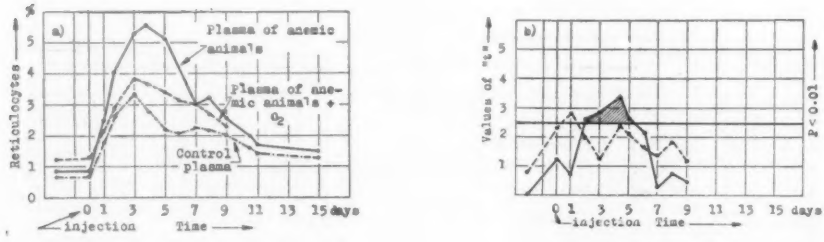

FIG. 4. — *Mean values for the percentage of reticulocytes of the control group (discontinuous line — o — o), the group injected with plasma of anemic rabbits, maintained free from contact with the air (continuous line ————) and the group injected with plasma of anemic animals submitted to the action of oxygen (discontinuous line — x — x). b) Values of "t" for the difference between the mean values for the percentage of reticulocytes of the control group, and the group injected with plasma of anemic animals, submitted to the action of oxygen (discontinuous line — o — o — o —). Values of "t" for the difference between the mean values for the percentage of reticulocytes of the group injected with plasma of anemic rabbits, maintained free from contact with the air, and the group injected with plasma of anemic animals submitted to the action of oxygen (continuous line ————).*

previous to the injection. Throughout the paper this group shall be referred to as group B_2.

Group C: Control receptors. — Eighteen rabbits of both sexes (average weight 1 533 g) were used. These animals received plasma intravenously (10 ml per kg of body weight) obtained from normal rabbits. This plasma was maintained free from contact with the air up to the moment of injection. This group shall be referred to as group C in this paper.

RESULTS

As can be seen in figs. 2 and 3 the degree of anemia obtained by the bleeding of the receptor animals was similar in groups B_1 and C ($P > 0.32$).

In fig. 1 it can be seen that the reticulocyte response of group B_1 was significantly greater ($P < 0.01$) than that of the control group C from the 1st to the 6th day after the injection.

The reticulocyte response of group B_1 (injected with anemic rabbit's plasma) was significantly greater ($P. < 0.01$) than that of group B_2 (injected with oxygenized anemic rabbit's plasma) from the 2nd to the 5th day, after the injection (fig. 4). There was a significant difference ($P < 0.01$) between the reticulocytes of group B_2 and C only on the first day after the injection (fig. 4).

During the course of the experiment, the mean values for hemoglobin were similar in groups B_1 and C (fig. 3). The mean values for erithrocytes of group B_1 showed however a significant drop ($P < 0.01$) on the 7th and 8th day after the injection (fig. 2).

DISCUSSION

The fact that the degree of anemia obtained by the bleeding on the day of the injection was similar in groups B_1 and C, indicates that in all probability the stimulus to erithopoiesis produced by this bleeding was of comparable intensity in these two groups. Therefore the greater reticulocyte response of group B_1, can be explained as an effect of the plasma of anemic animals. This greater reticulocyte response can be due either to an increased production of young red cells or to an insufficient ripening of the reticulocytes. This latter possibility seems remote in view of the great amount of reticulocyte ripening substance in the plasma of anemic animals ([8]).

The fact that one injection of plasma of anemic rabbits has no other effect on the peripheral blood picture than the production of an increased reticulocyte response, is in agreement with the work of Krumdieck ([7]). No explanation can be offered for the transient drop of the number of red cells in group B_1 which occurred after the value of reticulocytes had dropped to control level. The significantly smaller reticulocyte response of rabbits injected with plasma of anemic animals submitted to the action of oxygen, can be explained by the inactivation of the substance (or substances) present in the plasma of anemic rabbits, by this gas. The effect of oxygen on plasma of anemic rabbits would indicate that the substance or substances present have similar properties to that of the factor found in the blood of newborn babies ([6]).

It is a well-known fact that low oxygen tensions stimulate erithropoiesis and it has been shown recently that the breathing of mixtures with 50 % oxygen for several days, depresses erithropoiesis in the adult man (11). That the lack or excess of oxygen do not act directly, stimulating or depressing erithropoiesis, seems clear in view of the results obtained by Rosin (10) who has shown in bone marrow cultures, that low pressures of O_2 depress erithropoiesis, while high partial pressures of this gas have a stimulating effect. It has also been shown in animals, during severe maintained stimulation of erithropoiesis, that the oxygen saturation of bone marrow is within normal limits (5).

Since the action of oxygen on erithropoiesis cannot be explained as a direct influence on the bone marrow, it seems probable, as our experiments and those of Klingelhöffer (6) suggest, that it acts through a humoral mechanism which is influenced by variations in the partial pressures of this gas. The nature and origin of the humoral mediator are unknown. Work carried out in this laboratory has demonstrated that the activity of plasma of anemic rabbits, is probably not due to an increased amount of vitamin B_{12} or folic acid. Studies are now being carried out to determine in wihch fraction of the plasma of anemic rabbits resides its erithropoietic activity.

SUMMARY

1) The effect on erithropoiesis of plasma obtained from rabbits rendered anemic by bleeding is studied.

2) The plasma of anemic rabbits, maintained free from contact with the air, produced a significantly greater reticulocyte response ($P < 0.01$) than that produced by the injection of plasma of normal animals, in the receptor rabbits.

3) The plasma of anemic rabbits submitted to action of oxygen produced a significantly smaller reticulocyte response ($P < 0.01$) in the injected animals, than that produced by plasma of anemic rabbits, maintained free from contact with the air.

4) It is suggested that the action of oxygen on erithropoiesis is mediated through a humoral mechanism.

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FUNDAMENTAL BASIS OF THE SPECIFICITY OF PRESSOR AND DEPRESSOR AMINES IN THEIR VASCULAR EFFECTS

III. — The specific hypotensive action of some "pressor amines" (*)

F. HUIDOBRO AND R. CROXATTO

(Departamento de Farmacología y Laboratorio de Química Biológica, Universidad Católica de Chile. Santiago, Chile)

IN A PREVIOUS paper it was demonstrated that certain "pressor amines" did establish a blockade to the hypertensive effects of similar amines. If those results are interpreted through the physicochemical principles that command biological specificity (according to Pauling's ideas) it could be accepted that "pressor amines" are linked by surface complementarity to a hypertensive cellular receptor within the vascular territory (4). It was observed, during the experiments, that certain substances were able to modify such blockade. Of the studied substances, some "pressor amines" of aromatic ring, acetylcholine and histamine, proved to induce such effect. If these facts are also interpreted according to the physicochemical principles that command biological specificity —according to Pauling's suggestions (15)—, it must be admitted that certain portion of the molecule's morphology of histamine, acetylcholine and pressor monoamines of aromatic ring is common. Therefore, the respective cellular receptors may be occupied with certain statistical probability by these molecules.

The present paper deals with the pharmacological possibilities of modifying the blockade of the hypertensive and hypotensive receptor for "pressor amines", and to verify the hypothesis just indicated.

METHODS

One hundred and fifty two cats weighing from 1 500 g to 4 000 g were used All animals were anaesthetized with 1 cm^3 per kg of a solution containing 25 % urethane and 3.3 % of sodium pentobarbital ("Nembutal" Abbott) given intraperitoneally. Blood pressure was recorded by communicating the carotid artery to a mercury manometer. Sometimes the contralateral carotid was tied. Both vagi were sectioned in the neck. Some animals were atropinized with from 1 mg to 1.5 mg per kg of 1 % atropine sulphate "Merck" injected in the femoral vein. Some cats

(*) This work was financed in part by a grant from the Fundación Gildemeister, Santiago, Chile.

were injected with larger amounts of atropine (see Results). A cannula was placed in the trachea if artificial respiration was necessary. Respiratory movements were also recorded in some experiments.

The drugs used and the employed doses were as follows:

1) Acetylcholine hydrochloride (Hoffmann La-Roche) in doses of 0.02 mg.
2) Sodium nitrite (Barker's Analyzed) in doses of 2 mg.
3) Nitroglycerin (F. Ch. III ed.) in doses from 0.01 mg to 0.02 mg.
4) Histamine bi-hydrochloride (Hoffmann La-Roche) in doses of from 0.005 mg to 0.1 mg.
5) Barium chloride (Merck) in doses of 1 mg.
6) Pitressin (Pituitrol fuerte "Sanitas") in doses of 2 U.
7) Tetramethylammonium bromide (Sanitas) in doses of 10 mg given intraperitoneally.
8) Tetraethylammonium bromide (Sanitas) in doses of from 2 mg to 10 mg.
9) Hypertensin in doses of 2 U.
10) Dibenamine (N,N-dibenzyl-chlorcethylamine "Gibaudan Delawana Inc.") in doses of from 15 mg to 25 mg per kg, injected intraperitoneally 2 to 3 hours before the experiment.
11) Benadril (Parke & Davis, dimethylaminoethyl-benzydril ether hydrochloride), in doses of 5 mg per kg, injected in the femoral vein. Also, it was given intraperitoneally in doses of from 10 mg to 20 mg per kg.
12) Antistine ("Ciba", methanesulphonate of 2-phenylbenzylamine-methylimidazoline) in doses of from 2 mg to 4 mg per kg if given intravenously, and in doses of from 5 mg to 10 mg per kg if given intraperitoneally.
14) Vonedrine hydrochloride (1-methylamine-2-phenylpropane) in doses of 1 mg.
15) Phenylbutylamine hydrochloride in doses of 1 mg.
16) Tuamine sulphate in doses of from 1 mg to 2 mg.
17) Fouramine (2-amine-4-methylhexane) in doses of from 1 mg to 2 mg.
18) 2-methylamine-cyclopentyl-propane in doses of from 1 mg to 2 mg.
19) Amphetamine sulphate ("Smith, Kline & French Laboratories", phenyl-1-amine-2-propane) in doses of 0.5 mg.
20) Ephedrine hydrochloride (phenyl-1-methylamine-2-propane) of unknown origin (was bought in a pharmacy) in doses of 1 mg.
21) Propadrine base (phenyl-1-amine-2-propanol-1) in doses of 0.5 mg.
22) Phenylethylamine hydrochloride (Hoffmann La-Roche) in doses of 1 mg.
23) Phenyl-n-propylamine sulphate in doses of 1 mg.
24) Neo-synephrine hydrochloride (metahydroxyphenyl-1-methylamine-2-ethanol-1) in doses of 0.02 mg.
25) Tyramine hydrochloride ("Eastman Kodak Co.", p. hydroxyphenyl-2-amine ethane) in doses of 1 mg.
26) Paredrine hydrobromide (p. hydroxyphenyl-1-amine-propane) in doses of 0.4 mg.
27) Adrenaline hydrochloride (Clin. commercial solution) in doses of 0.01 mg.
28) Cobefrine hydrochloride (3,4-dioxyphenyl-1-amine-2-propanol) in doses of 0.04 mg.
29) Epinine hydrochloride (3,4-dioxyphenyl-1-methylamine-2-ethane) in doses of 0.04 mg.
30) Arterenol monhydrate bitartrate (3,4-dioxyphenyl-1-amine-2-ethanol) in doses of 0.01 mg.
31) Butanefrine hydrochloride [1 (3,4-dioxyphenyl)-2 amine-1-butanol] in doses up to 0.5 mg.

32) Isuprel sulphate [1(3,4-dioxyphenyl)-2-isopropylamine-ethanol] in doses up to 0.5 mg (*).

All drugs were dissolved in distilled water and injected intravenously, unless otherwise specified. Volumes injected never exceeded 0.2 ml.

RESULTS

I. Modifications of the blockade of the hypotensive and hypertensive cellular receptors of "pressor amines"

1) *Hypotensive substances*:

A) *Sodium nitrite and nitroglycerine.* — These substances neither modify tachyphylaxis induced by amphetamine, ephedrine, phenylethylamine and propadrine, nor the response of any other drug injected during the course of thachyphylaxis.

B) *Acetylcholine.* — Acetylcholine was injected to animals under tachyphylaxis induced by "pressor amines" of bencenic ring, i.e., when these amines did not induce hypertension or when they produced hypotension. Once hypotension after acetylcholine was over, the monoamine under study was injected again. Using ephedrine, it was observed (in 3 animals out of 10) that the drug induced hypotension or did increase hypotension present before acetylcholine was given. Something similar happened with amphetamine (in 5 experiments out of 10) and with phenylethylamine (in 2 experiments out of 5). It must be noted that this phenomenon is not constant (it cannot be easily repeated in the same animal) and that hypotension induced or increased by the drug after acetylcholine is slight. This phenomenon was not observed if atropine was previously injected.

C) *Histamine.* — This drug proved to be a more potent agent in reproducing the above described phenomenon: it appears more intense with longer duration and may be repeated several times in the same animal. It was observed using tyramine (in 7 experiments), ephedrine (in 5 experiments), amphetamine (in 8 experiments) and phenylethylamine (in 10 experiments) (see fig. 1). If amphetamine was injected alternatively with histamine, statistical analysis indicated that histamine did not diminish the quantities of monoamine necessary to induce tachy-

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(*) Aromatic pressor amines are racemic except arterenol which is levogyrate. Drugs 24, 27, 28, 29, 30, 31 and 32 were stabilized with sodium bisulphite in such concentration that the final solution was in a concentration of 1 %. To preserve the solutions, a drop of merthiolate (Eli Lilly & Co.) was added for every 20 ml.

We are grateful to Sharp & Dohme for the donation of 22; to Parke & Davis for 21; to Smith, Kline & French Laboratories, through the courtesy of Dr. M. L. Moore, for 26; to Dr. D. F. Marsh for 15; to Winthrop Stearns Inc. for 24, 29, 30 and 31; to The W. M. S. Merrel Co. for 14; to Eli Lilly & Co., through the courtesy of Dr. E. E. Swanson, for 16, 17 and 18; to the Instituto Bioquímico Beta for 32.

All these generous gifts allowed the possibility of doing this work.

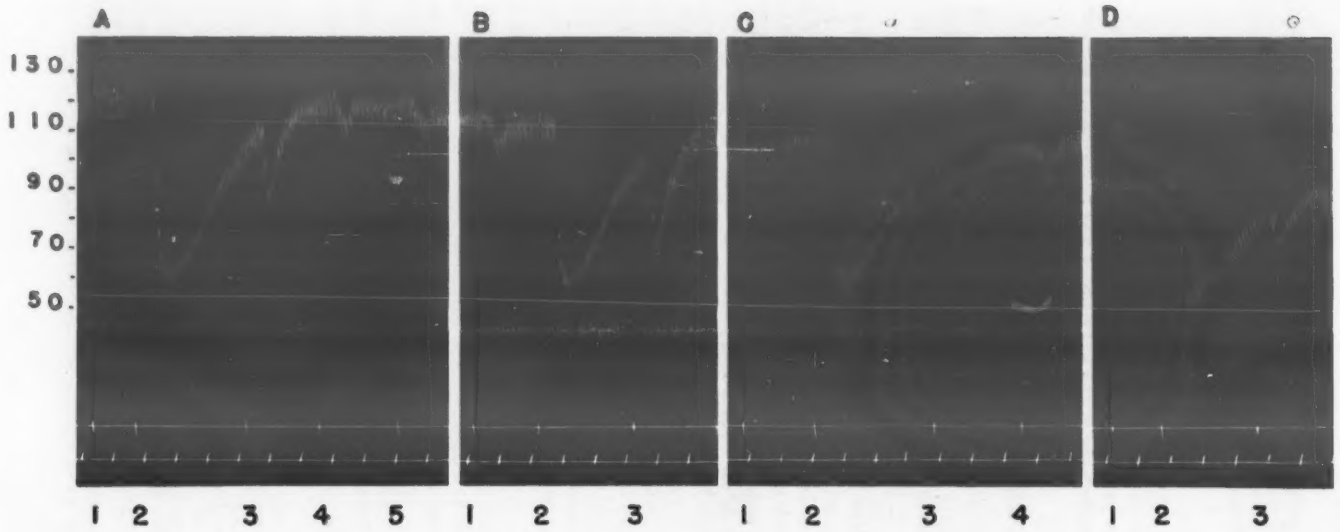

FIG. 1. — *The increased hypotension induced by bencenic "pressor amines" (the animal being under tachyphylaxis to their hypertensive action) after histamine. A) 1, 3, 4, 5: ephedrine, 1 mg; 2: histamine, 100 micrograms. B) 1 and 3: 0.5 mg of amphetamine; 2: 100 micrograms of histamine. C) 1, 3, 4: 1 mg of phenylethylamine; 2: 100 micrograms of histamine. D) 1 and 3: tyramine, 1 mg; 2: 100 micrograms of histamine. Time in minutes.*

phylaxis. In this case, average values of amphetamine were (1.00 ± 0.2 mg), which have no significative difference with the controls (1.90 ± 0.31 mg). Also, it was intended to know whether histamine was able to potentiate the hypotensive action of catecholic ring amines. To study this point, adrenaline and dibenamine were used. As it is well known, adrenaline causes hypotension in animals previously injected with dibenamine (13). This effect does not increase after histamine. Also, it was thought of interest to learn if hypertension induced by animes of bencenic

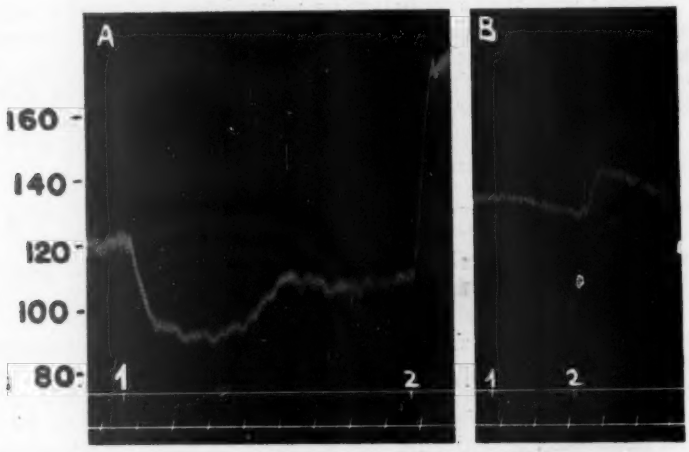

FIG. 2. — *Waning of hypotensive effect of TEA determined by amphetamine. A) 1: 7.5 mg of tetraethylammonium bromide. 2: 1 mg of amphetamine. B) 1: 7.5 mg of tetraethylammonium bromide. 2: 1 mg of amphetamine. Between A and B: 10 minutes. Time in minutes.*

or phenolic ring, after dibenamine (5), could be transformed into hypotension by the action of histamine. This was not the case, for histamine does not modify the response to "pressor amines", in animals that previously received dibenamine. The hypotensive effect of nitroglycerine does not change after dibenamine and histamine maintains its hypotensive action, even though it has a lesser hypotensive action (12).

D) *Tetraethyl ammonium bromide and tetramethyl ammonium bromide.* — Tetraethyl ammonium bromide may diminish tachyphylaxis to certain drugs (14). Thus, it was employed to study its action on tachyphylaxis to "pressor amines". Also, tetramethyl ammonium bromide was used. Amphetamine and tetramethyl ammonium bromide were injected alternatively or amphetamine was administered to animals that previously received tetramethyl ammonium bromide (5 to 10 minutes before) intraperitoneally. The results of series of six animals were statistically analyzed, and no significative difference was observed between this series and a control series in which amphetamine caused tachyphylaxis.

Using tetraethyl ammonium bromide an interesting result was obtained. Acheson and Moe demonstrated that hypotension caused by the tetraethyl ammonium bromide ion may be exactly reproduced each time the drug is given (1). On the contrary, it has been observed that tetraethyl ammonium bromide used alternatively with amphetamine, gave different results, i.e., the hypotensive effect of tethaethyl ammonium bromide rapidly disappears (fig. 2). Randall, Peterson and Lehman (16) found that ephedrine antagonizes tetraethyl ammonium bromide effects. Our results could be explained through the work of Luco, Martorell and Reid (6) who found that amphetamine potentiates the action potentials of sympathetic post-ganglionic fibers. This observation was interpreted as caused by ganglionic discharges provoked by amphetamine by increasing ganglionic excitability. Thus the ganglionic blocking power of tethaethyl ammonium bromide vanishes.

2) *Hypertensive substances:*

Several drugs were studied, after the hypertensive responses to paredrine, ephedrine, amphetamine, phenylethylamine and tyramine were blocked by previous administration of "pressor amines", i.e., after tachyphylaxis to this substance was induced.

A) *"Pressor monoamines" of catecholic ring.* — If adrenaline is injected —after tachyphylaxis to a "pressor amine" of bencenic or phenolic ring has been induced— it is observed that a drug previously without effect on blood pressure determines hypertension. This action may last from 1 to 3 minutes, and also may be repeated at will. This phenomenon was observed when adrenaline was employed in animals under tachyphylaxis to tyramine (in 3 out of 3 experiments); when propadrine was employed in one experiment; also, using ephedrine (in 14 animals out of 14); amphetamine (in 10 out of 12 animals) (fig. 3); phenylethylamine (in 4 out of 5 experiments) (fig. 3). Something similar happens if the animal becomes tachyphylatic to ephedrine, and if cobefrine is used instead of adrenaline. In three experiments, the injection of ephedrine that followed cobefrine administration induced a rise in blood pressure. The same is true for arterenol (in 3 out of 3 experiments). Tyramine was employed once and turned the normotensive response of ephedrine into an hypertensive one. ,

The alternative administration of adrenaline and amphetamine demeures tachyphylaxis. Results statistically analyzed indicated that a total dose of 1.90 ± 0.31 mg of amphetamine was needed to induce tachyphylaxis on the condition that single doses be of 0.5 mg each (4). If this dose (0.5 mg of amphetamine) ir alternatively employed with 20 micrograms of adrenaline, 3.70 ± 0.37 mg of amphetamine would now induce tachyphylaxis (6 aminals). There was significative difference. Thus, it is possible to conclude that adrenaline is antagonistic to tachyphylaxis induced by amphetamine. In other words, a larger dose of amphetamine is necessary to induce tachyphylaxis under this condition.

B) *Other hypertensive substances: pitressin, hypertensin, and barium chloride.* — The above described phenomena were not reproduced if instead of using adrenaline, cobefrine, arterenol or tyramine, other hypertensive substances like pitressin, hypertensin and barium chloride were employed. In this series, 15 animals were used.

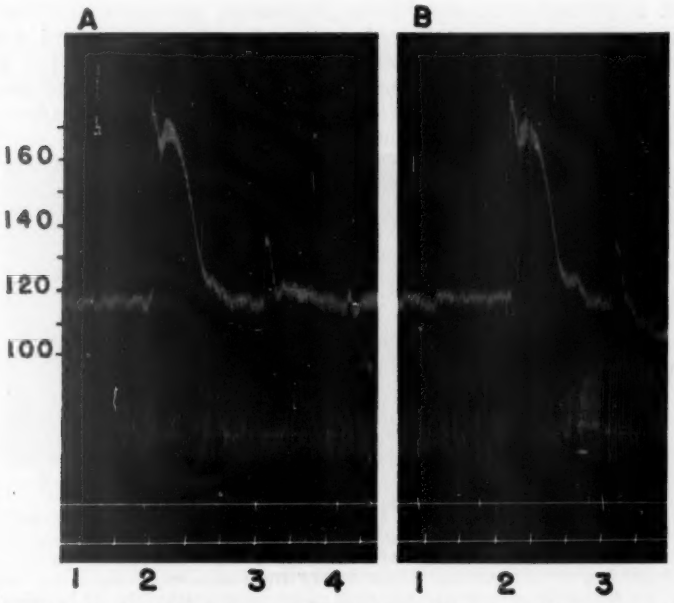

FIG. 3. — *The effect of adrenaline upon tachyphylaxis to the effects of amphetamine and phenylethylamine. A) 1, 3, 4: 0.5 mg of amphetamine. 2: 20 micrograms of adrenaline. B) 1, 3: 1 mg of phenylethylamine. 2: 20 micrograms of adrenaline. Time in minutes.*

II. Partial similarities of the molecular morphology of histamine, acetylcholine and "pressor amines"

The experiments described in section I induced us to think that the molecular morphology of histamine, acetylcholine and "pressor amines" was partially similar in what morphological structure is concerned. Thus, these molecules would occupy at once —according to probability— the vascular receptors that are specific for these substances. The following experiments proved this hypothesis.

1) *Effect of anti-histaminic substances on the depressor action of "pressor amines", and on acetylcholine effects.*

The anti-histaminics employed were benadril, antistine and neoantergan. Dosage and administration routes are already indicated (see

Methods). These substances decreased or abolished the hypotensive effect of 5 micrograms of histamine, given intravenously. If under this condition "pressor amines" are injected (tachyphylaxis was previously induced) the hypotension that might have been produced was largely reduced. Figure 4 shows clearly the typical effect of benadril on hypotension caused by amphetamine and phenylethylamine. Anti-histaminics diminish hypotension caused by amphetamine, phenylethylamine, vonedrine, phenylbutylamine, ephetonine, tuamine, fouramine and 2-amine-cyclopentyl-propane. This inhibition is not equaly intense for all the studied substances. Tuamine and 2-amine-cyclopenthylpropane are most

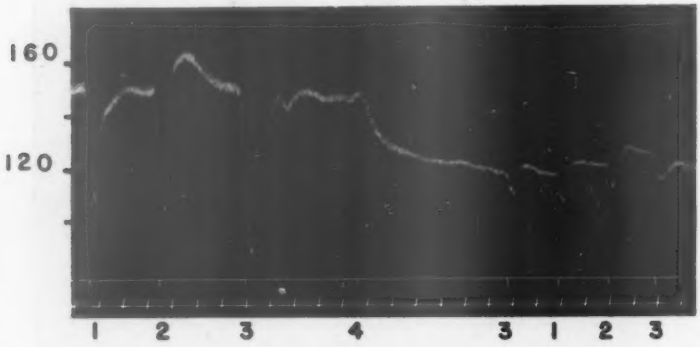

FIG. 4. — *Partial obliteration of the hypotensive action of amphetamine and phenylethylamine by benadril. Cat weighing 2000 g, anaesthetized with sodium pentobarbital. The animal had received previously 2 mg of amphetamine. 1) 2 mg of amphetamine. 2) 4 mg of amphetamine. 3) 5 micrograms of histamine hydrochloride. 4) benadril, 20 mg per kg given intraperitoneally. Time in minutes.*

resistant. Some authors have not found this effect of anti-histaminic drugs ([10]).

Contrary to what has been described, anti-histaminics may greatly intensify the action of "hypotensive amines", chemically related to "pressor amines". Thus, butanefrine and isuprel increased their hypotensive effects after the injection of anti-histaminics (fig. 5), agreeing with what was found for isuprel by other authors ([11]). Also, it was thought of interest to see whether the anti-histaminics could increase the hypotensive actions of adrenaline, epinine, cobefrine, arterenol and neosynephrine, because these substances compete for the same cellular receptor of butanefrine and isuprel ([5]). These experiments were done in animals that previously received dibenamine. After a control injection, where adrenaline and other amines of catecholic ring were given, an anti-histaminic (antistine) was injected. Under these conditions, it was observed that the anti-histaminic substance increased the hypotensive action of adrenaline and cobefrine (fig. 6). Also, if epinine (given previously to antistine) did not induce hypotension, an hypotensive

effect appeared (fig. 6). As it has been described before ([5]), small doses of cobefrine, arterenol and neosynephrine —after dibenamine— always induce hypertension. Antistine considerably diminished hypertension induced by these substances. It has been described that benadril may potentiate the hypotensive effect of adrenaline ([7]). Because in the literature there is plenty of evidence about the atropinic effects of antihistaminics ([7, 8, 9]) those experiments have not been repeated.

2) *Action of atropine on the effects of histamine on the depressor action of "pressor amines".*

Atropine (atropine sulphate "Merck") in doses of from 3 mg to 10 mg per kg was injected intraperitoneally. This route of administra-

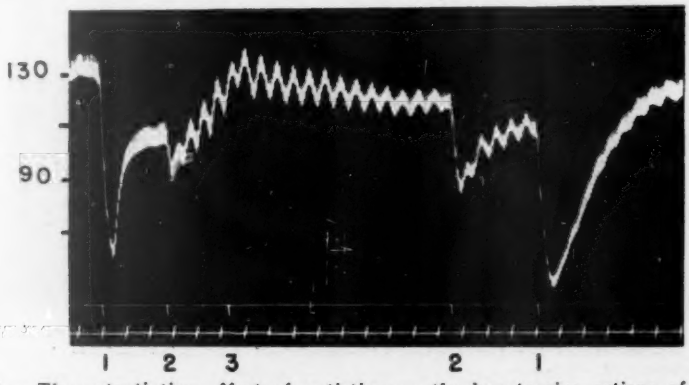

FIG. 5. — *The potentiating effect of antistine on the hypotensive actions of isuprel and butanefrine. 3 600 g cat, anaesthetized with sodium pentobarbital. 1) 0.5 mg of isuprel. 2) 4 micrograms of butanefrine. 3) 7 mg of antistine given intraperitoneally. Time in minutes.*

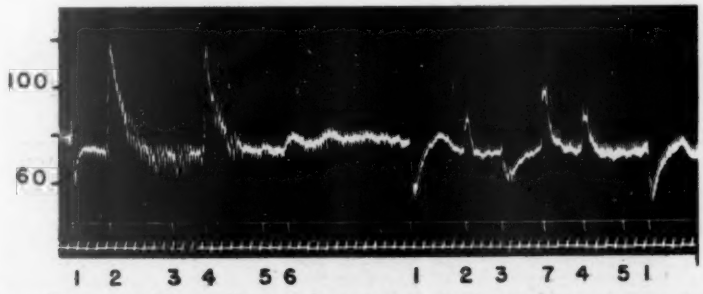

FIG. 6. — *The potentiating effect of antistine on the hypotension of some "pressor-amines" of catecholic or phenolic structure, after dibenamine. 2 700 g cat anaesthetized with sodium pentobarbital. 15 mg of dibenamine, per kg, 3 hours before the experiment. 1) 1 microgram of adrenaline. 2) 40 micrograms of cobefrine. 3) 40 micrograms of epinine. 4) 10 micrograms of arterenol. 5) 20 micrograms of neo-synephrine. 6) 7 mg of antistine, per kg, given intraperitoneally. 7) 80 micrograms of arterenol. Time in minutes.*

tion was used in order to disminish as far as possible the hypotension that is determined by the drug. This effect is more marked after larger doses (10 mg). The actions of atropine on the hypotensive effects of histamine, amphetamine and phenylethylamine were studied, thus, it may be seen in figure 7 that atropine reduced the hypotensive effects of histamine and of "pressor amines". The anti-histaminic effects of atropine have been described elsewhere ([8]).

3) *The effects of substances that block the depressor action of "pressor amines" on the actions of histamine and acetylcholine*

It was demonstrated in a previous paper that dibenamine impeded the appearance of the depressor effects of "pressor amines" ([5]). Also, it has

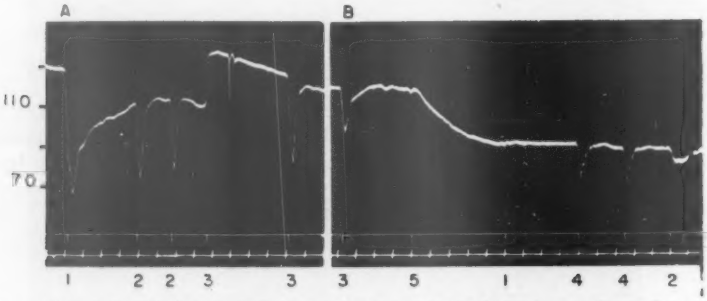

FIG. 7. — *Decrease of the hypotensive effects of both histamine and amphetamine by the action of atropine. Cat weighing 2 600 g anaesthetized with sodium pentobarbital. 1) 10 micrograms of acetylcholine hydrochloride. 2) 5 micrograms of histamine hydrochloride. 3) 1 mg of amphetamine. 4) 2.5 mg of amphetamine. 5) 10 mg of atropine sulphate given intraperitoneally. Between A and B, 10 minutes and two injections of 1 mg of amphetamine. Time in minutes.*

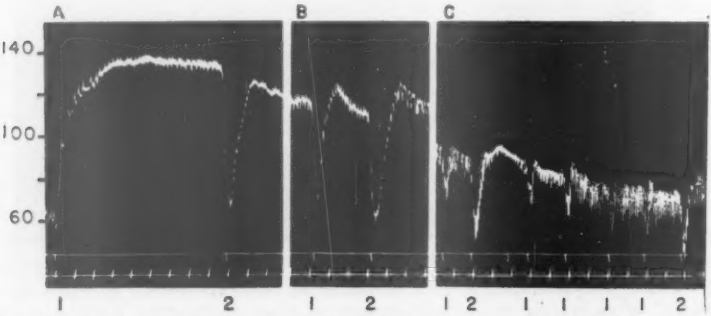

FIG. 8. — *Decrease of the hypotensive effect of histamine by repeated doses of amphetamine. Cat weighing 3 100 g anaesthetized with sodium pentobarbital. 1) 2 mg of amphetamine. 2) 5 micrograms of histamine hydrochloride. Between A and B, 18 minutes and five injections of 2 mg of amphetamine. Between B and C, 18 minutes and seven injections of 2 mg of amphetamine. Time in minutes.*

been pointed out that dibenamine has an anti-histaminic action (12). It is well known that amphetamine may induce tachyphylaxis to hypotension (4). If acetylcholine or histamine are injected under this condition, the hypotensive responses of these drugs are smaller than if injected before tachyphylaxis was induced (fig. 8). Usually this action is not marked. Also it has been found that a "pressor amine" ("ortoxine") has an anti-histaminic action (3).

Discussion

All the employed drugs may alter blood pressure through several mechanisms. We interpreted these phenomena as a direct action of the drugs on the smooth muscles of blood vessels (4).

It was found that the anti-histaminic drugs are quite adequate to study the probable morphology of cellular receptors that would be specific for "pressor amines" in their hypotensive effects. The hypotensive action of some "pressor amines" is either antagonized or potentiated by anti-histaminics. Some would be linked to one vascular receptor by surface complementarity, and the others to some other vascular receptors. "Pressor amines" can be displaced from the cellular receptors by "pressor amines" of catecholic ring. This action seems to be specific for the latter structure because other substances such as pitressin, hypertensin, barium chloride or other "pressor amines" do not have this action. If these phenomena are interpreted through the physico-chemical principles that command specificity (15), it must be accepted that amines of catecholic ring displace the amines of bencenic ring, because the former have larger surface complementarity with the receptor. The same explanation could be given as an interpretation for the increased hypotension determined by amines of bencenic ring after histamine or acetylcholine that seems to be a specific phenomenon. If this postulate is correct it is required to accept the idea that certain "pressor amines" induce hypotension through a linkage to the adequate receptors for histamine because "pressor amines" and histamine do have at least in part, a common morphology. The pharmacological effects of histamine —referred to hypotension induced by pressor amines— are partially similar to the effects of acetylcholine; also, it is evident that part of the molecular morphology of histamine and "pressor amines" is similar to that of acetylcholine. Such similarity would allow the linkage of the molecules of "pressor amines" (by surface complementarity) to the histaminic and acetylcholinic receptors, according to statistical possibilities. In such case, the molecules of histamine could be anchored to the receptor for acetylcholine, and vice-versa. To accept this explanation, it would be necessary to accept that if "pressor amines" do occupy the cellular receptors for histamine and for acetylcholine, they must reduce the effects of both histamine and acetylcholine. Furthermore, it must be accepted that anti-histaminics do have a structural morphology that is complementary to that of the vicinity of the receptor, they would

determine thereby a blockade by interposition. The effects of dibenamine could be interpreted in a similar way. It follows, therefore, that the hypotensive effects of acetylcholine and of "pressor amines" must be reduced by an anti-histaminic agent. Also, in a similar way, atropine should partially block the hypotensive effects of "pressor amines" and of histamine. All these hypothesis were confirmed in this paper.

When a "pressor amine" is injected, its molecules would be linked simultaneously —according to diverse statistical probabilities— to the specific hypertensive receptor, to the histaminic receptor and to the receptor for acetylcholine. Further, if the molecule has a phenolic or catecholic structure, it also may be anchored to the specific hypotensive receptor of "depressor amines", chemically related to "pressor amines". If a "depressor amine" of a given chemical morphology is injected, the possibilities of being anchored to a given number of receptors are greater, but its probabilities are different, depending on the degree of complementarity. In other words, they would be linked in different proportions to their specific hypotensive receptor, to the specific hypertensive receptor for "pressor amines" and to the specific receptors for histamine and acetylcholine ([8]).

It is understood that in this discussion, only the pharmacological effects related with blood pressure were considered.

All the possibilities mentioned above, do explain certain pharmacological characteristics such as: the potentiation of the effects of sympatomimetic substances by anti-histaminic agents ([8, 9]); that certain substances block both the adrenergic and histaminic effects; dibenamine that blocks the adrenergic effects, impedes the hypotensive actions of some "pressor amines" by incomplete blockade of the hypotensive receptor ([5]). Also, it has an anti-histaminic effect, probably due to an incomplete blockade of the histaminic receptor ([12]). Other substances could act by the same mechanisms ([8, 9]). Furthermore the increased hypotension determined by "depressor amines" (chemically related to "pressor amines") under the effect of anti-histaminic agents, is clearly understood.

SUMMARY

Adult cats, anaesthetized with sodium pentobarbital were used. Both vagi were cut in the neck. Some animals were atropinized. Blood pressure was recorded by connecting the carotid artery to a mercury manometer. Thus, a number of drugs were studied on their effects on blood pressure.

When tachyphylaxis to tyramine, ephedrine, propadrine, phenylethylamine and amphetamine was induced, the reinjection of any of these drugs after a single dose of adrenaline, determined hypertension. This phenomenon was present if amines of catecholic ring were employed. It did not happen after any other hypertensive drugs.

Histamine and sometimes acetylcholine increased hypotension induced after "pressor amines" had been repeatedly injected. This phenomenon did not appear if other hypotensive agents were used.

The necessary dose of "pressor amines" in order to induce tachyphylaxis was increased by adrenaline. Histamine did not determine the opposite action.

Histamine did not increase hypotension induced by adrenaline after dibenamine. Also, it did not provoke the appearence of the hypotensive effects of "pressor phenylamines" after dibenamine.

Under tachyphylaxis to the hypertensive action of "pressor amines" (amphetamine, phenylethylamine, vonedrine, phenylbutylamine, ephetonine, tuamine, fouramine and 2-amine-cyclopentyl-propane), the injection of an anti-histaminic agent such as benadril, neoantergan or antistine, blocked the hypotension that might have resulted after the reinjection of any of the "pressor amines".

It was confirmed that the studied anti-histaminic agents have an atropin-like effect.

Atropin has an anti-histaminic action and blocks hypotension induced by amphetamine and phenylethylamine.

A direct relationship between tachyphylaxis to the hypotensive effects of amphetamine and histamine was observed.

Anti-histaminic agents potentiate hypotension induced after butanefrine. Dibenamine increased the hypotensive action or decreased the hypertensive effects (it depended on the substance or the dose) of amines of catecholic ring.

The results obtained are interpreted through the physico-chemical principles that command biological specificity. According to Pauling's ideas, some "pressor amines" would act as depressor substances because of surface complementarity with the histaminic receptor and in a lesser degree with the acetylcholine receptor. This possibility would indicate that the molecules of histamine, acetylcholine, "pressor amines" and "depressor amines" do have a partially similar morphology.

Amines of catecholic ring may also be anchored to the specific hipotensive receptor of "depressor amines", chemically related to "pressor amines", and obviously to the specific hypertensive vascular receptor of "pressor amines".

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NEUROMUSCULAR ACTION OF THE CROTALUS TERRIFICUS TERRIFICUS (Laur.) POISONS *

AVELIOO BARRIO (H.) AND OSNALDO VITAL BRAZIL

(Instituto Malbrán, Ministerio de Salud Pública de la Nación and Instituto de Fisiología, Facultad de Ciencias Médicas de Buenos Aires)

STUDYING the action of different samples of venom of *Crotalus terrificus*, proceeding from various parts of Argentina, our attention was called by the discordance between the symptoms that we observed during this poisoning and the descriptions of the clinical picture as well as the neuromuscular action of said poisons, made by various authors who have worked on this problem.

Vital Brazil, 1914 ([31]) and his schoo! consider the venom of *Crotalus terrificus* as a paralyzing neurotoxin. Houssay and Pavé 1922 ([18]), after investigating the action on batrachians maintain that all the ophidic poisons are curarizing including those of the Crotalidae family. Later on, Houssay and Hug, 1928 ([16]), find that the venom of *C. terrificus* has a central action and therefore in the classification of Houssay, 1930 ([15]), on the physiological action of these poisons, this venom is considered as a neurotoxin, separating it from the true curarizing poisons (*Naia*, etc.).

O. Vital Brazil, 1945 ([32]), not finding an antagonizing effect between prostigmin and the crotalic Brasilian poison, on pidgeons, says that the paralysis can not be attributed to a true curarizing action.

Vellard, 1945 ([3]), recognizes the paralyzing action but minimizes the clinical importance, attributing to shock the cause of death.

Other authors that have investigated the neuromuscular action of the North American *Crotalus* poisons, maintain that they are not paralyzing, for example: Arthus ([2]), Kellaway and coworkers ([19]), Essex and Markowitz ([11]), and limit the activity of said poisons to their shocking effect.

With all these antecedents, we resolved to investigate the neuromus-

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* Paper read at the 11th Communications Meeting of the Instituto Malbrán on December 13, 1949. Afterwards new experiments were added made at the Instituto de Fisiología de la Facultad de Medicina de Buenos Aires, also at the Instituto Malbrán del Ministerio de Salud Pública and at the Departamento de Farmacología da Faculdade de Medicina da Universidade de São Paulo.

cular action of the *Crotalus terrificus* poison, using samples from different parts of Argentina, Brasil and other American countries.

MATERIAL AND TECHNIQUES

The neuromuscular action of the *Crotalus terrificus* venom was investigated using samples removed and kept separated according to their origin: *

Argentina: poisons removed at the Instituto Malbrán of specimens from Salta, Jujuy, Santiago del Estero, Tucumán, Catamarca, Santa Fe, Chaco, Formosa, Córdoba, Corrientes and Misiones.

Brasil: poisons from São Paulo, Paraná, Minas Gerais, Mato Grosso and Goyaz.

Paraguay: poison from San Pedro.

Bolivia: poison from Santa Cruz.

Costa Rica: (Crotalus terrificus durissus) Poison from San José.

Also the poisons of *Bothrops alternata, B. jararaca* and *B. jararacussu,* from specimens from the Instituto Malbrán were investigated as a means of comparison.

The symptoms of poisoning in rats weighing 180 to 200 g were observed, and the isotonic contractions of the *soleus-gastrocnemius* were recorded. The technique used was the same as that of previous works ([33, 34]), with the only difference that anaesthesia was not used.**

On most of the experiments, the poison was injected by intraperitoneal route (0.5 to 2 mg) and when the symptoms were very apparent the nerve and muscle were prepared for the record. Maximal unipolar stimulation of the muscle was always made, either directly or indirectly. In all cases artificial respiration was applied in the final periods. Moreover, the rat's sciatic nerve was isolated and immersed in solutions of different concentrations of crotalic poison. Also, the classic experiments of C. Bernard to demonstrate the mechanism of action of curare were repeated on batrachians.

Besides, the contractions of the isolated gastrocnemius of batrachians were recorded.

The following substances, dissolved in physiological serum, were used to investigate their effects on the neuromuscular action of the poisons.

Adrenaline hydrochloride	0.1 %
Atropine sulphate	0.1 %
Calcium chloride	50 %
Quinidine sulphate	20 %
Magnesium sulphate	10 %
Prostigmin sulphate	0.05 %
N. metil-O-metil-bebeerina chloride	0.02 %
Chloral hydrate	10 %
Nembutal	2 %

RESULTS

The systematic study of the neuromuscular action of these neurotropic crotalic poisons has enabled us to distinguish two different types, namely Type I (spasmodic and veratrinic) and Type II (paralyzing and neurotoxic) these types corresponding to a certain geographical distribution which will be discussed later when dealing with geographical variations.

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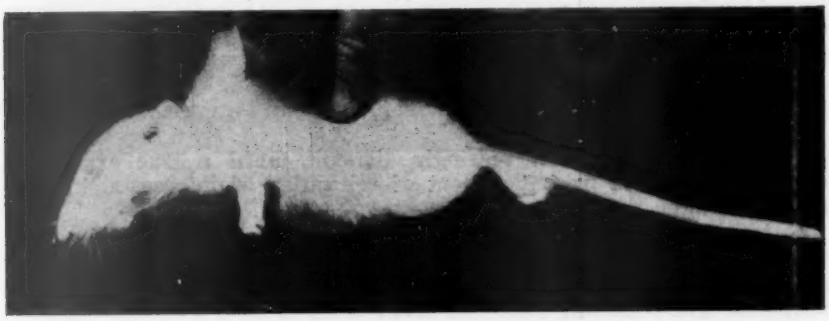
* We are thankful to Dr. Eduardo Vaz and Wolfgang Bücherl, of the Instituto Butantan; to Dr. Aníbal Pereira, of the Instituto Pinheiros and Dr. Alfonso Trejos W., Chief of laboratory of Hospital San Juan de Dios, for supplying samples of venom.

** Anaesthesia is not necessary because the crotalic poison seems to deaden the sensibility.

Type I

Symptoms. — The syndrome provoked by *Crotalus terrificus* venom coming from Santiago del Estero, Argentina, is very typical: in the rat, where the symptoms are more apparent, constant and regular, two well defined periods or phases are distinguished: 1) phase of spasmodic seizures or tonic convulsions; 2) phase of hypotonia and paralysis.



a

b

FIG. 1. — *Poison of* Crotalus terrificus, *type I (origin: Santiago del Estero, Argentina). Injection of 1 mg by intraperitoneal route in a rat of 150 g of body weight. a) Phase of the spasmodic seizures or tonic convulsions 15 minutes after injection. Note the rigidity of the entire body, the spasm of the orbicular of the eyelids, the clenched fore paws and the stiffening of the hind legs and tail. b) Hypotonia and paralysis phase 45 minutes after injection. The spasms have ceased; the body, head, tail and legs hang flaccid, the eyes remain open (this same flaccid aspect is observed at the beginning in the rat poisoned with crotalic venom type II).*

After a passing period of excitement, one of the most typical signs of the poisoning appears: one or both of the hind legs become rigid in extension when attempting to walk. These spasm stops momentarily, but as time passes, these attacks become much more intense and prolonged appearing with great frequency, almost without interruption. Simultaneously, the fore legs contract spasmodically, presenting the characteristically claw hand; the eyes closing each time more intensely and frequently until a true blepharospasm is constituted. Later on, when the animal is lightly stimulated or tries to move, tonic convulsions appear spreading like a wave to all muscles; the animal becomes rigid, the body extended, the eyes closed, the fore legs in flexion, the hind legs and tail extended (see fig. 1, a).

Breathing becomes increasingly difficult, until a true expiratory dysnea is produced by impediment of relaxation of intercostal muscles and diaphragm.

After certain time, the duration of which is in inverse relation with the dose of poison injected, the spasmodic seizures decrease in intensity and frequency. On injecting new doses of poison, this first phase fails to reappear. The poisoning continues progressing in an irreversible manner.

The second phase begins with the disappearance of the spasms; the eyes open, breathing becomes partially normal, and the animal is able to execute some voluntary movements of slight duration and amplitude; on account of being in an asthenic and hypotonic state it cannot execute efficient contractions. The rat has in this phase (see fig. 1, b) a typical flaccid appearance, with the head, the four legs and the tail hanging limp. At the same time, breathing movements become shorter and more superficial. If the dose of the poison is lethal, the paresis and hypotonia progress; breathing becomes insufficient and imperceptible; temperature decreases and death by anoxia results, with great cyanosis and asphyxiating convulsions. The heart continues to beat for several minutes after breathing stops. This explains why artificial respiration often prolongs life for some time.

If the dose given is sub-lethal a slow recuperation starts in which the second phase of paresis and hypotonia may be prolonged for several days accompanied by light spasms; apparently a partial concomitance of both phases occurs, with preponderance of the second one. These symptoms are seen in all their details in mice also.

The dog goes through the two phases described, with the difference that its legs are permanently extended, giving the animal the appearance of standing on four rigid and inarticulated legs.

The guinea pig and the rabbit do not close their eyelids and the stiffness of their hind legs is discrete and not always constant; nevertheless, its movements are clumsy due to difficult muscular relaxation in the first phase and in the second one to paralysis.

The batrachians *(Bufo arenarum* and *Leptodactylus ocellatus)* are very resistant to the poison; only with large doses and after several hours is it possible to obtain results. If 5 mg are injected into the dorsal sac, the hypotonic phase takes place after 8 to 10 hours; similar facts were proved by Houssay and Pavé ([18]), also by Essex and Markowitz ([11]).

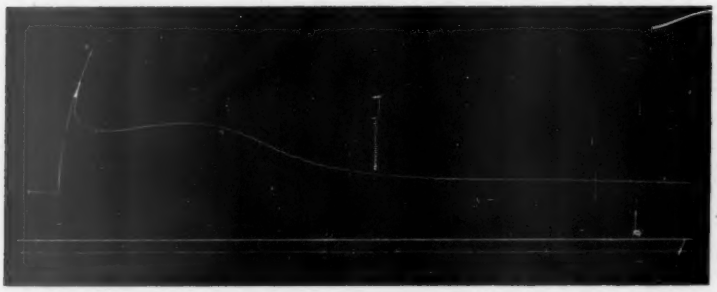


FIG. 2. — *Contraction of the rat's* soleus-gastrocnemius *produced by a single stimulation of the sciatic with a 5 volts current. Observed 25 minutes after the i.p. injection of 5 mg of* Crotalus terrificus *poison type I (origin: Santiago del Estero). Note the normal contraction followed by a slower relaxation, in the middle of which another slow contraction begins before reaching the base line. (Rat not anesthetized; time: intervals of 6 seconds).*

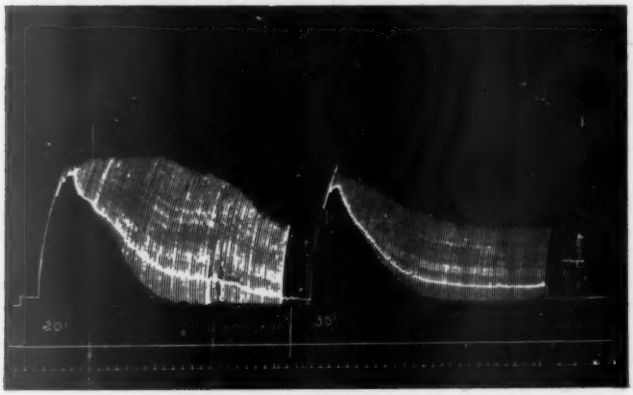


FIG. 3. — *Contraction of the* soleus-gastronemius *of the unanesthetized rat. At 0 minutes, 0.5 mg of* Crotalus terrificus *poison type I (origin: Santiago del Estero) were injected i.p., 20 minutes after the sciatic stimulation is started with 30 stimuli per minute and 5 volts of intensity. The first stimulus causes a normal contraction but as the muscular relaxation is slowed, the following impulses find the muscle in nearly complete contraction. As the stimulation continues, relaxation increases until at the end of the first series, an almost normal curve is obtained. Leaving in repose for 7 minutes and applying the stimulation again, a similar record is obtained, i.e.: typical veratrinic response at the beginning, and fatigue of veratrinic effect afterwards. (Time: intervals of 6 seconds).*

Nevertheless, giving greater quantities of venom (more than 50 mg) through the intravenous or intra-sinusal route, at the end of some thirty minutes the spasmodic phase appears, followed later on by the paralytic phase.

Neuromuscular effects, myographic records. — Fibrillary contractions of exposed muscles are observed in the rat, under the effects of the poison.

The myograms obtained during the first phase of the poisoning are very typical and agree completely with the symptoms observed in the intact animal. It can be observed that under either direct or indirect

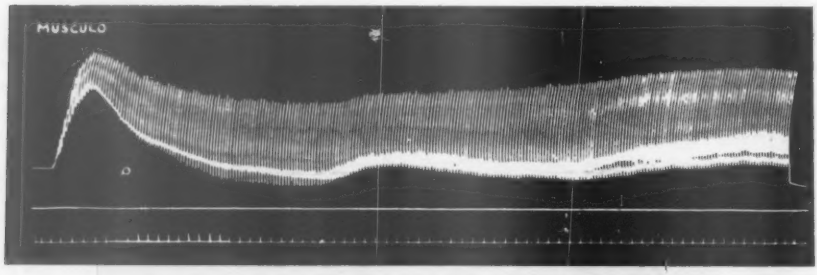


FIG. 4. — *The same preparation as that of fig. 3, with the difference that the stimulus is applied directly to the* soleus-gastrocnemius. *Record taken 40 minutes after the injection of the poison. The veratrinic effect can be observed, at the beginning; later on, the effect disappears. Nevertheless, at 3 and 6 minutes, two phases of little intensity (veratrinic-like response) are observed. (Time: intervals of 6 seconds).*

stimulation employing the most diverse frequencies, the muscle contracts normally, except that its relaxation is difficult and takes place very slowly as in the veratrinic poisoning (see figs. 2 and 5) with the only difference that the amplitude of the contractions is not sensibly increased as happens in the last case.

On the rest the analogies of both poisonings are very numerous: slow partial relaxation, followed by a secondary contraction before final relaxation (fig. 2); repeated direct or indirect stimulation provokes the phenomenom of fatigue seen in veratrinic poisoning (Bezold and Hirt [6], Carvallo and Weiss [8]), the effect reappearing after a short rest (fig. 3) or even in a less noticeable form during prolonged stimulations (fig. 4) as has been observed by Carvallo and Weiss ([8]) in the rabbit.

Apart from fibrillation, spontaneous contractions are observed in preparations with sectioned nerve and without any external stimulation similar to those noted by Bottazzi ([7]), Laporta ([22]) and Stozharov ([27]) with veratrine. At other times, it is enough to stimulate any part of the body, as we have already mentioned in the intact animal, in order to obtain a veratrinic contraction of the muscle prepared for recording, with

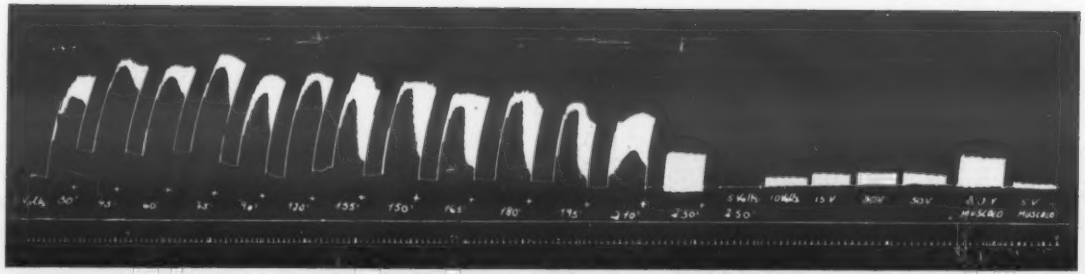


FIG. 5. — *Stimulation of the sciatic with 30 volleys a minute at the beginning, with 5 volts. At 0 minutes 0.7 mg of* Crotalus terrificus *poison type I (origin: Santiago del Estero) are injected i. p. Note the veratrinic response that may always be observed by allowing the muscle to rest between each series of stimulations. The crosses indicate that the kymograph stops working. At 230 minutes the veratrinic response has disappeared. At 250 minutes, the muscle does not respond to the stimulation of the sciatic with 5 volts. The intensity of the stimulus is then increased to 10, 15, 30 and 50 volts, and the response to indirect stimulation reappears. If the stimulation is then applied directly to the* soleus-gastrocnemius, *a greater response is observed with 5 to 50 volts. (Rat not anesthetized. Time: intervals of 6 seconds).*

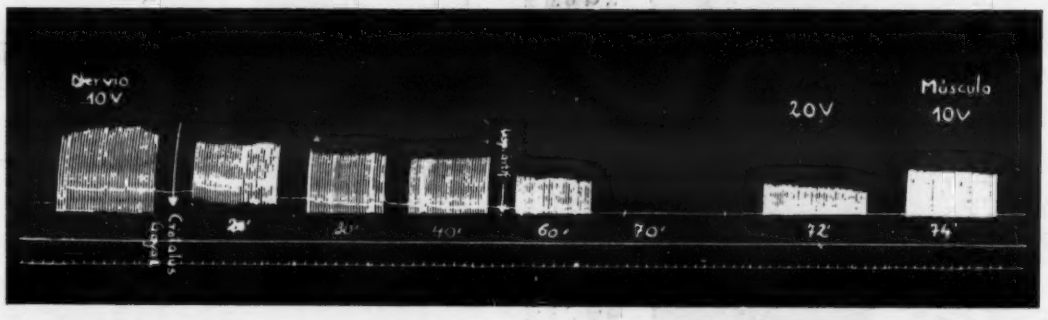


FIG. 6. — *Stimulation of the sciatic with 30 volleys per minute and 10 volts at the beginning. At 0 minutes, 1 mg of* Crotalus terrificus *poison type II (origin: Goyaz, Brasil) was injected i.p. At 20 minutes the magnitude of the contraction has diminished and* veratrinic response is not observed. *At 55 minutes, artificial respiration is applied and apnea appears. At 60 minutes the amplitude of the contraction is greatly reduced and at 70 minutes the muscle does not respond any more to indirect stimulation with 10 volts. The intensity is then increased to 20 volts and at 72 minutes a muscular response is again obtained. At 74 minutes* the soleus-gastrocnemius *is directly stimulated with 10 volts and a response is obtained even if there was no response when indirect stimulation was applied with the same voltage. (Rat not anesthetized. Time: intervals of 6 seconds).*

sectioned nerve; this propagation of the stimulus will be further dealt with in the discussion. The veratrinic type curve is obtained by direct stimulation of the muscle whose nerve has been cut fifteen days before (fig. 7) or after the injection of curare (fig. 8) as was observed by Back and Brown (4), Fontes (13) and Querido (25) with veratrine. These results make it clear that the action of the *Crotalus terrificus* poison acts directly on the muscle, and exclude the participation of the intramuscular nerve fibers and myoneural junctions in the phenomenom, although they

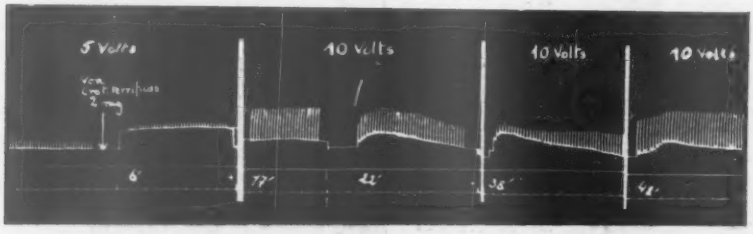


FIG. 7. — *Direct stimulation of the* soleus-gastrocnemius; *preparation with nerve sectioned 15 days before. At 0 minutes, 2 mg of* Crotalus terrificus *poison type I (origin: Santiago del Estero) are injected i.p. At 6 minutes 30 stimuli (5 volts) per minute are applied and after 17 minutes, 10 volts are used. In all cases a veratrinic response appears. (Rat not anesthetized. Time: intervals of 6 seconds).*

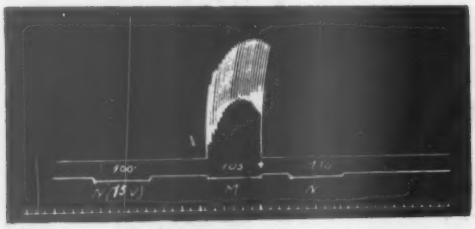
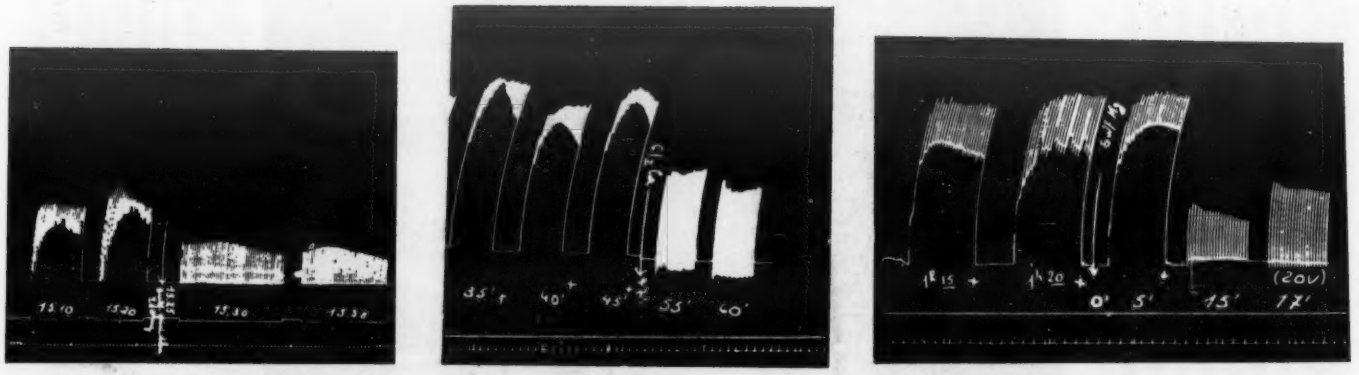


FIG. 8. — *At 0 minutes 0.5 mg of* Crotalus terrificus *poison type I (origin: Santiago del Estero) were injected, i.p. At 30 minutes 0.3 mg of N-metil-O-metil-bebeerina (curare) was injected by i.p. route. At 100 minutes it was observed that the gastrocnemius did not respond to indirect stimulation with 15 volts, due to complete block produced by curare. At 105 minutes, beginning direct stimulation the typical veratrinic response appears (the cross indicates that the kymograph has been stopped). At 110 minutes the sciatic nerve is again stimulated showing that the muscle does not yet respond. (Rat not anesthetized; time: intervals of 6 seconds).*

do not exclude the possibility that the poison may have some independent effect on the nerve fibers.

During the first phase of the poisoning, some drugs of known antagonistic action to veratrine were tested. Quinidine sulphate, calcium chloride and magnesium sulphate injected by intraperitoneal route normalize the muscular response altered by the crotalic poison (fig. 9, a, b,



a *b* *c*

FIG. 9. — *Effects of some substances of antiveratrinic action on the crotalic poison action. Rats* soleus-gastrocnemius *contractions after stimulation of the sciatic with 5 volts. Rats were injected with 1 mg of* C. terrificus *poison type I (origin: Santiago del Estero) by i.p. route. The crosses indicate that the kymograph was stopped. (Rats not anesthetized; time: 6 seconds intervals). a)* Rat 1: *45 minutes after the injection of crotalic poison 200 mg of quinidine sulphate were injected by i.p. route. 5 minutes afterwards the veratrinic response disappeared. b)* Rat 2: *47 minutes after giving the crotalic poison, 200 mg of calcium chloride was injected i.p.; note the disappearance of the veratrinic response in 8 minutes. c)* Rat 3: *One hour and twenty five minutes after the injection of crotalic poison, 100 mg of magnesium sulphate were injected i.p. producing disappearance of the veratrinic response in 15 minutes. At 17 minutes the muscle was directly stimulated by applying 20 volts, obtaining a greater no veratrinic response.*

c). Atropine sulphate gave negative results. On the contrary, chloral hydrate and nembutal diminished or abolished the repetitive response, either to the direct or indirect stimulation of the muscle (fig. 10, a, b). Querido ([25]) found that ether and choloroform inhibit the repetitive response, but not so urethane. In regard to the evolution of the *Crotalus terrificus* poison (origin Santiago del Estero) it is observed that like in poisoning by veratrine, the muscular relaxation after a period in which

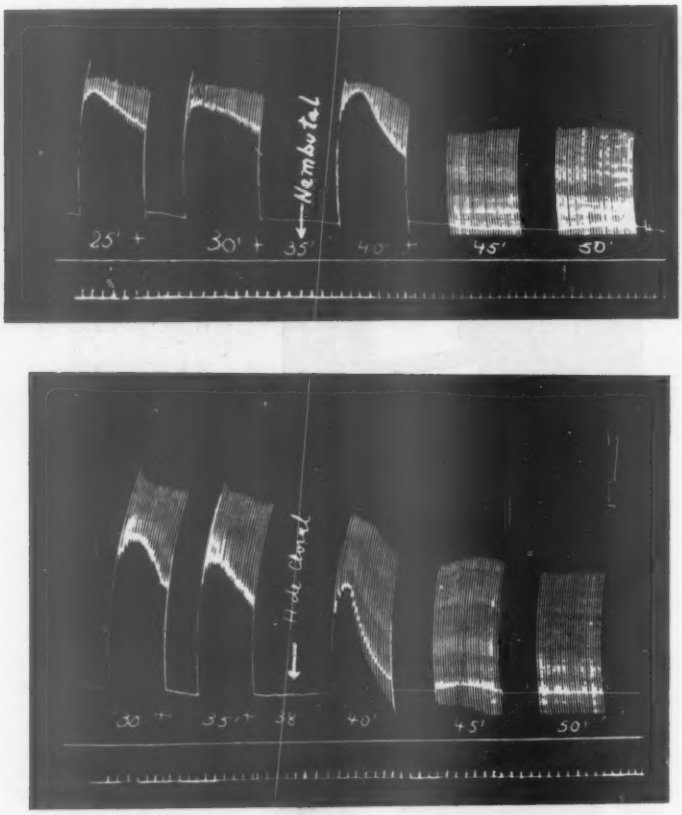


FIG. 10. — *Effect of the anesthetics on the veratrinic response of the crotalic poison. Myograms of the* soleus-gastrocnemius *of rats not anesthetized, obtained by stimulation of the sciatic with 5 volts, 30 stimuli per minute. In both cases, at 0 minutes, 1 mg of* C. terrificus *poison, type I (origin: Santiago del Estero) is injected by i.p. route. The crosses indicate that the kymograph has been stopped. (Time: intervals of 6 seconds). a)* Rat 1: *At 35 minutes, 18 mg of nembutal is injected i.p.; 10 minutes after the injection, the veratrinic response had disappeared. b)* Rat 2: *At 38 minutes, 100 mg of chloral hydrate was injected i.p. no veratrinic response is observed 7 minutes after the injection.*

this altered, is temporarily reestablished and the paralysis phase due to blockade of the nerve impulses begins (figs. 5, 11). At first, a response is obtained (figs. 5, 11) by increasing the stimulation, but when poisoning advances the loss of nervous excitability is complete for any voltage. At this moment if the muscle is directly stimulated it responds. It presents a phenomenom similar to curarization which differs however in some particulars.

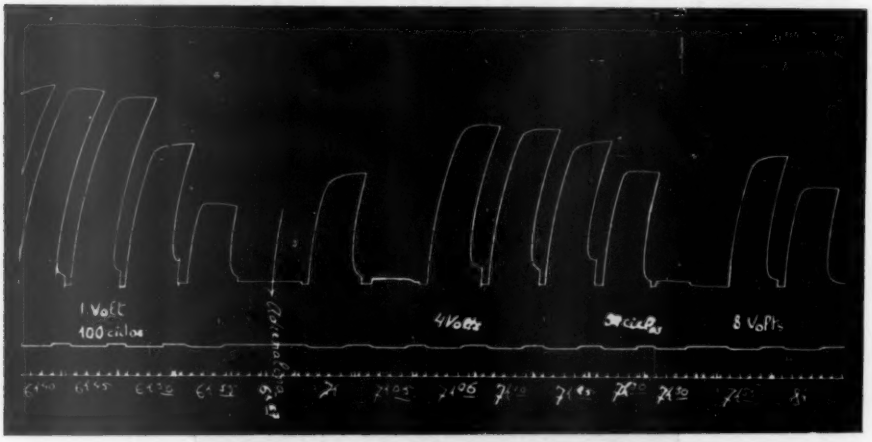


FIG. 11. — Crotalus terrificus *poison type I (origin: Santiago del Estero). Contraction of the* soleus-gastrocnemius *by stimulating the sciatic with tetanic frequencies. Observations made during the hypotonia and paralysis phase. Rat weighing 200 g, not anesthetized, injected at 5 p.m. with 0.5 mg of poison by i.p. route; at 6.45 p.m., practically no veratrinic response is obtained stimulating with 1 volt and 100 cycles. At 6.55 p.m., lower contraction* without Wedensky inhibition *phenomenon, even though stimulation was of high frequency. At 6.57 p.m. 0.3 mg of adrenaline chlorhydrate, is injected i.p. and 3 minutes later (7 h. p.m.) a slight increase in the contraction is observed. At 5 minutes, there is almost no response. At 7.06 p.m. the stimulation is increased to 4 volts noting a high increase in the contraction.* Inhibition phenomena are not observed. *At 7.30 p.m. 50 cycles are applied and at 7.50 p.m. there is practically no muscular response to stimulation. At 7.55 p.m. stimulation is increased to 8 volts and the response reappears. (Time: intervals of 1 second).*

With *Crotalus* poison (origin, Santiago del Estero), even during advanced periods the phenomenom of Wedensky inhibition is not seen. as long as the preparation is not fatigued (fig. 11). Moreover, prostigmin neither increases nor restores the indirect excitability.

Once the period of repetitive responses has disappeared, new injections of poison do not succeed in reproducing the phenomenom, indicating the evolutive and irreversible character of this poisoning. With veratrine, Carvallo and Weiss [8] observed that if the first injection does not produce the typical effect at the start, additional new doses will not succeed in provoking it.

The batrachians are resistant to crotalic poison, but if the gastrocnemius of a toad *(Bufo arenarum)* or of a frog *(Leptodactyllus ocellatus)* is prepared and immersed in a 0.15 % solution of type I crotalic poison a veratrinic response is obtained. Moreover, we repeated in these batrachians the C. Bernard experiments for curare already made by Houssay *et al.* ([17]) with ophidic poisons, arriving to the same conclusion, i.e: the paralysis is not due to the action of the poison on the nerve trunks.

Besides, we observed that the isolated sciatic of the rat, immersed in solutions of crotalic poison does not lose the excitability and shows only some muscular fibrillation.

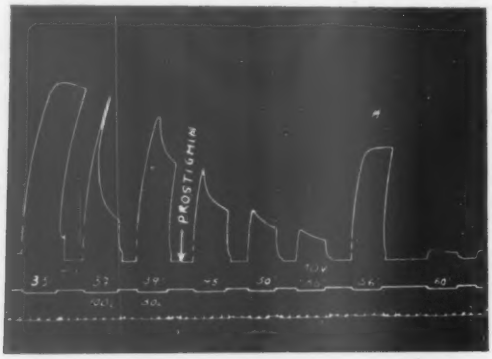


FIG. 12. — C. terrificus *poison type II (Origin: Goyaz, Brasil).* Soleus-gastrocnemius *contractions by stimulation of the sciatic with tetanic frequencies. At 35 minutes,* a light Wedensky inhibition *is noted. At 37 minutes the frequency is increased to 100 cycles and the inhibition is greater. At 39 minutes the frequency is reduced to 50 cycles and it is observed a corresponding decrease of inhibition. At 40 minutes, 0.25 mg of prostigmin are injected by i.p. route. At 45 and 50 minutes, it is observed that this drug does not show in this case, desinhibitory action. At 55 minutes, 10 volts are applied to stimulate the nerve (due to the advanced stage of the poisoning no increase of the contractions is observed as a result of this increased stimulation). At 56 minutes, 10 volts are applied directly to the muscle; a great contraction is obtained, which also decreases later on. (Time: intervals of 1 second).*

Type II

Symptoms. — The syndrome provoked by *Crotalus terrificus* venom coming from Minas Gerais, Brasil, differs greatly from the former one. It begins with the second phase of hypotonia and paralysis, without passing through the state of spasmodic accesses and tonic convulsions. The rat arrives progressively at a state of muscular flaccidity. It rarely suffers a complete paralysis, and generally, even being hypotonic, it still conserves some muscular force, especially in the legs. Simultaneously, alterations in the rythm and amplitude of its breathing appear; dyspnea gradually increases, until breathing stops after asphyxial convulsions. At

this moment reflexes are present and the oculopalpebral reflex persists even some hours after breathing stops. The heart, as in case type I, continues to beat for a while; artificial respiration also prolongs life for many hours.

Neuromuscular effects, myographic records. — The myoneural action is manifest in an advanced stage of poisoning, generally after breathing stops; from which it may be concluded that at the beginning, hypotonia and paralysis are not of peripheral origin. The first phenomenom observed is the Wendensky inhibition, which appears when 100 stimuli per second are used and is less marked if 50 stimulations per second are used (see fig. 12). This increases with the evolution of the poisoning and cannot be atrributed to fatigue, as no desinhibitory effects can be observed after a prolonged rest. The amplitude of the contractions decreases gradually until finally a spontaneous partial loss of the inhibition is observed thus arriving at a complete block. In very advanced stages when stimulation is increased, no response appears, but if the stimuli are directly applied to the muscle, this contracts normally (fig. 12), there is a loss of indirect excitability as with type I of this poison. Prostigmin, in contrast with what we have observed with Elapidae poison ([33, 34]) has no desinhibitory action, does not restore indirect excitability and has no effect on the breathing movements.

GEOGRAPHICAL VARIATIONS

The *Crotalus terrificus* poison presents the most interesting and important geographical variations.

The changes in its chemical constitution and its pharmacological properties have been the object of study by many authors. Vellard ([29]), Amaral ([1]) and Picado ([24]) studied the variations of its neurotoxic, proteolytic and shocking properties; one of us (A. Barrio [5]) investigated the variations of its contents of riboflavin and l-ophi-aminoacid-oxidase.

Here we have dealt with the differences of neuromuscular action of *C. terrificus terrificus* poisons proceeding from Argentina, Brasil, Bolivia, Paraguay and Costa Rica with the following results: all the poisons from Argentina (origin: Jujuy, Salta, Tucumán, Catamarca, Santiago del Estero, Córdoba, Santa Fe, Corrientes, Chaco, Formosa, Misiones); Paraguay (San Pedro) and Bolivia (Santa Cruz) belong to type I (spasmodic and veratrinic).

The brasilian poisons belong to type I (spasmodic and veratrinic) and to type II (paralyzing and neurotoxic) with the following approximate distribution: Paraná, type I; São Paulo, both types, with predominance of type II in the southern and northeastern regions, and type I preferably distributed in the northern and northeastern regions of said state. In Goyaz type II is prevalent with a geographical dispersion not fully known as yet, while until now only type II was found in Minas Gerais and Matto Grosso. It should be noted that all these poisons were extracted separately from each specimen and kept separated, thus avoiding the

possibility of error, as it must be taken into account that both types may exist in neigbouring regions. No differences were observed in the spasmodic intensity or in the veratrinic effect of type I poison. The type II poisons injected in large doses (5 to 10 mg i. p.) never provoked spasms thus demonstrating that they lack the characteristic factor of type I.

The poison of Costa Rica origin constitutes really another sub-type, but as it belongs to another subspecies (*Crotalus terrificus durissus*) it will be considered apart. This poison does not provoke spasms like type I, but neither does it determine a pronounced and precocious hypotonic state, as does type II. It is very similar to the bothropic poisons, not only because of its proteolytic and shocking properties but also because of its neuromuscular action. We have found that the symptomatology and the myograms obtained, are similar to those observed with *Bothrops alternata, B. neuwiedii* and *B. jararacussu* poisons; certainly a state of flaccidity and paralysis finaly results; but once the apnea is set, artificial respiration prolongs life only for a short time. Symptoms of shock, hypotension and heart failure are evident, which cause the death of the animal. Therefore, we believe that in the Costa Rican crotalic poison and in the bothropic poisons, the paralyzing principle is present, although not in the proportion found in the South American *Crotalus* poison, and also we believe that it is masked by other intercurrent toxic factors which affect the circulatory system.

Discussion

According to our observations and to the reported similarity shown i. e. : slow and partial relaxation followed by a slow secondary rise and fall, spontaneous contractions, tendency to generalization, fatigability, effects of the antagonists, etc., we believe that the neuromuscular action of *Crotalus terrificus terrificus* (Laur.) poison type I is very similar to that of veratrine, with the differences mentioned above.

While the veratrinized muscle responds with a contraction of much greater amplitude than the normal one, the response of the muscle under the effect of crotalic poison is equal to, or scarcely greater than the normal one. Moreover, batrachians (which as is well-known, are very susceptible to the action of *Veratrum* alkaloids) are very resistant to the action of the crotalic poison. Nevertheless, the similarity is notorious and even both phases of the poisoning can be compared with what is observed with veratrine: repetitive response at the start, followed by loss of excitability.

For this reason we propose to classify this poison as "veratrinic", at least with reference to its muscular action, in the same way as we call curarizing other animal venoms which produce effects similar to those of this vegetable poison. This would be the second animal venom classified as veratrinic; the first one is the scorpionic poison which Houssay ([14]), and Del Pozo ([10]), classified as belonging to this type.

The propagation or generalization of the spasm to other skeletal muscles while the animal is stimulated, and the appearance of sponta-

neous contractions without the intervention of any external stimulus, deserves due consideration. An explanation of these phenomena could be the interaction of the muscular fibers, as observed by Wyss ([35]) with veratrine, or the interaction of the nerves fibers, according to the opinion of Feng and Li ([12]). Nevertheless, we are inclined to think of the presence of some stimulating substance which once liberated would cause spontaneous contractions and later on their generalization. This hypothesis is supported by the fact that we have obtained spontaneous contractions in denervated preparations, a circumstance that excludes all nervous participation in the origin of the phenomenom. Besides, our opinion is reinforced by the experiments of Szent-Gyorgyi, Bacq and Goffart ([28]), who obtained without previous stimulation the typical repetitive response on the hind legs of the veratrinized batrachian, by the sole perfusion of said hind legs with a perfusion fluid proceeding from another similar preparation on the moment of the contraction. According to Bacq ([3]) and Rosenblueth ([26]), the liberated substance is potassium.

As already mentioned, the action of the crotalic poison type II and type I in its second phase is not absolutely the same as that of curare and of the poison of the Elapidae. In effect, although these substances block indirect stimulation when the muscle still responds to direct stimulation, many facts show they are different.

If the action of crotalic poison and veratrine were similar, both substances should exert their action on the same anatomical elements. It has been demonstrated that veratrine acts independently on the muscle and on the nerve but does not interfere with neuromuscular transmission. For this reason, the veratrinic curarization proposed by Lapicque ([21]) is not accepted. It is known that the substances that affect the neuromuscular synapse produce changes on the end plate potential; veratrine does not affect this potential ([20]). Moreover, veratrine does not change the sensitivity of the skeletal muscles to acetylcholine ([9, 23]) as occurs with curare.

With crotalic poison type I, as in the case of veratrine, neither curare nor nervous degeneration impede the repetitive response of the muscle under the effects of this venom; thus, it must be admitted that the poison acts directly on the muscle.

As we showed before with type I poison, if during an advanced state, when the muscle does not respond to the indirect maximal stimulation, the voltage is increased, the response reappears. This fact suggests a decrease of the nervous excitability rather than a neuromuscular block.

On the other hand, it must be noted that prostigmin, a drug which antagonizes the action of curare and which also counteracts the curarizing effect of the Elapidae ([34]) in no case restores or increases the indirect excitability, nor improves the dyspneic breathing of the poisoned animals. Neither does it abolish the Wedensky inhibition caused by type II poison.

In spite of what we have reported above, the results we have obtained immersing sciatic nerves of the rat and batrachians in different solutions

of crotalic poison, in which we could never observe a disappearance or decrease of the indirect excitability, we are obliged to reject the possibility that the poison acts on the nerve trunks and admit a neuromuscular peripherical block that is different from the typical curarization for the above mentioned reasons. This conclusion is supported by the results obtained in repeating with crotalic poison on bratrachians the classic experiments of C. Bernard, for the curare, which are in complete accordance with those of Houssay and Pavé ([18]) made with ophidic poisons. Perhaps by recording the nerve and end plate potentials this problem may be solved.

We have not found an absolute coincidence between the hypotonia and paralysis phase of the intact animal poisoned by *Crotalus* venom and the period of loss of nervous and muscular excitability. This makes us think that the late appearing neuromuscular block, has little effect on the physiopathology of the syndrome, especially of that produced by crotalic poison type II, the muscular hypotonia and respiratory paralysis being almost certainly of central origin.

The fact that nembutal and chloral hydrate, considered as having central action only, are capable of producing a decrease or disappearance of the repetitive response in the crotalic-type I poisoning on preparations with sectioned nerve, leads us to suppose that these anaesthetics excercise some nervous and muscular action which has not yet been taken into account.

We point out the importance of the geographical variations, not only from the philogenetic and zoogeographic point of view, but because they contribute to the understanding of the pharmacodynamic mechanism of crotalic poison.

This suggests that the poisons of the Crotalidae family contain two different principles or factors. The veratrinic principle, which produces the repetitive response is exclusive of *Crotalus terrificus terrificus* type I. The paralyzing and neurotoxic factor would exist in variable concentrations in all the species studied of the Crotalidae family *(Crotalus terrificus terrificus,* types I and II, *C. terrificus durissus, Bothrops alternata, B. neuwiedii, B. jararaca, B. jararacussu,* etc.). The loss of excitability must be fundamentally attributed to this factor. It is very probable that the blockade caused by the crotalic-type I poison is due in part to the veratrinic factor, as veratrine also produces this phenomenom. Anyhow, the loss of excitability would explain why poisoning continues its irreversible course and why once the paralytic period is set, the administration of new quantities of poison does not provoke any more spasms. With the crotalic type I poison, in contrast to veratrine ([8]), even if given in large quantities, the spasmodic phase always appears preceding the paralytic one and the greater the dose, the shorter each phase becomes.

SUMMARY AND CONCLUSIONS

1) The symptoms of poisoning in the rat and other animals by *Crotalus terrificus terrificus* (Laur.) venoms are described. Simultaneously, the isotonic contraction of the *soleus-gastrocnemius* by direct and indirect stimulation of the muscle was recorded. According to the results obtained these venoms may be classified in two types: Type I (spasmodic and veratrinic), Type II (paralyzing and neurotoxic).

2) Type I. — This crotalic poisoning goes through two well defined periods or phases: a) phase of spasmodic seizures or tonic convulsions; b) phase of hypotonia and paralysis.

In the myogram, two phases are also observed: a) phase of normal contractions followed by slow relaxation (repetitive response). *The curve observed is similar to that produced by veratrine.* b) Phase of loss of indirect and afterwards direct excitability of the muscle. By increasing the intensity of the stimulus, reappearance of the response is generally obtained.

3) Type II. — The poisoning shifts directly towards the second phase. Muscular hypotonia and paralysis are observed. The myogram shows signs of a Wedensky inhibition first, then loss of excitability.

4) Several substances of recognized antiveratrinic action were tried (quinidine sulphate, magnesium sulphate and calcium chloride) which proved able to antagonize the effects of the first phase of poisoning type I, by causing the disappearance of the veratrinic response. Chloral hydrate and nembutal were shown to possess the same action.

5) Curarization and nervous degeneration do not impede the repetitive response under the effects of crotalic type I poison nor under the action of veratrine.

6) Crotalic poison type I and II, do not determine the loss of nervous excitability; however, the block they provoke is not identical to that observed with curare or with Elapidae poison, as prostigmin does not increase or restore the indirect excitability of the muscle nor improves the dyspneic respiration, nor causes the disappearance of the Wedensky inhibition produced by type II poison.

7) Nevertheless, an absolute coincidence between the hypotonic and paralytic phases of the poisoned intact animal and the phase of loss of excitability has not been found. Rats in a complete state of flaccidity and paresis responded with a normal contraction to indirect stimulation. This makes us suppose that apart from the causes of peripheral origin the central action of the poison plays an important part in the physiopathology of paralysis.

8) Attention is called to the importance of geographic variations, not only from the philogenetic and zoogeographic point of view, but also because they contribute to the clear understanding of the pharmacodynamic mechanism of crotalic poison.

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ESTUDIO COMPARATIVO DE LAS ACCIONES DEL DIAL, NEMBUTAL, PENTOTAL, CLORALOSA Y URETANO, SOBRE ELECTROCARDIOGRAMA, PRESIÓN ARTERIAL, RESPIRACIÓN Y ACTIVIDAD DE LAS ZONAS CARDIOAÓRTICA Y SENOCAROTÍDEA

POR R. ALVAREZ-BUYLLA

(Laboratorio de Electrofisiología. Escuela Nacional de Ciencias Biológicas, I.P.N. — México, D.F.)

UNO de los problemas que surgen al iniciar un trabajo de fisiología experimental es el de la selección de anestésico. Si se estudia la bibliografía correspondiente para buscar en ella bases que permitan una elección bien fundamentada, se encuentra gran cantidad de datos analíticos difíciles de sistematizar, ya que sobre determinados puntos existe una verdadera acumulación de trabajos, mientras sobre otros, a pesar de ser de importancia primordial, apenas se han realizado estudios.

Bourne, Bruger y Dreyer [13], Adolph y Gerbasi [2], Searles y Essex [64], Schendorf [62], Jarcho [44], Bollman, Svirbely y Mann [10], Hausner, Essex y Mann [32], Hamlin y Gregersen [31], Lockard [52], Bonnycastle [12], Martin y Rovertson [54], Poderman, McCarrell y Beecher [58], Barton, Wicks y Livingstone [8], Wakim [70], Penrod y Hegnauer [57], Smith y Oster [66], Flinker y McCarrell [19], Courtice [15] y otros han estudiado el efecto que ejercen distintos anestésicos sobre la hemoconcentración. Las conclusiones a que llegan son casi concordantes; las escasas divergencias encontradas parecen deberse a la utilización de animales de distintas especies.

Otro problema planteado en un número considerable de trabajos es si los barbitúricos ejercen o no, influencia inhibidora sobre las terminaciones del vago en el corazón. [Schaefer y Underwood [65], Lieb y Mulinos [50], Garry [20], Swanson [68], Rosenblueth y Simeone [60]. Rosenblueth y Rioch [61], Koppanyi y Linegar [45], Linegar, Dille y Koppanyi [51], Gruber [22], Gruzit, Dox, Rowe y Dodd [28], Gruber, Haury y Gruber [23], Gruber, Haury y Gruber [24], Gruber, Gruber y Colosi [26], Gruber, y Keyser [25], Walcott y Deyrup [72]]. Las conclusiones, en este problema, son a menudo contradictorias, pues unos autores afirman que los barbitúricos inhiben o paralizan el vago periférico, y otros lo niegan. Hay quienes localizan la acción de los barbitúricos sobre el mismo miocardio (Krayer, [46]), otros lo hacen sobre ganglios parasimpáticos intracardíacos (Linegar, Dille y Koppanyi, [51]), en tanto que Gruber, Haury y Gruber, [23, 27]) afirman que tiene lugar sobre las fibras pre y post ganglionares del vago.

Sin embargo, como ya hacían notar Comroe y Schmidt, son pocos los trabajos cuyos datos permiten comparar entre sí la influencia que cada anestésico ejerce sobre la presión arterial, respiración, función cardíaca y actividad de los reflejos propioceptivos que regulan la presión arterial.

Aunque en los trabajos de Vercauteren [69], Heymans y Bayles [39], Nowak [55], Donnatelli [18] y Abbate [1] se hacen estudios comparativos de la acción de diversos anestésicos sobre la presión arterial, respiración, función cardíaca y reflejos reguladores, en ellos no se incluyen los que más se utilizan en la actualidad en los laboratorios de fisiología y farmacología. Heymans y Bayles [39], Vercauteren [69] dan datos sobre la cloralosa, pero no hay posibilidad de compararlos con otros homólogos correspondientes al nembutal, dial y uretano. Por estas causas nos decidimos a realizar un trabajo con suficiente número de experimentos en perros y gatos, cuya finalidad fuese obtener cifras medias de los niveles de presión arterial y frecuencias cardíaca y respiratoria, así como disponer de otros datos que permitieran comparar el nivel funcional de los reflejos que tienen su origen en las zonas reflexogénicas cardioaórticas y senocarotídeas durante la acción de los anestésicos que, como el uretano, cloralosa, dial, nembutal y pentotal se utilizan con mayor frecuencia en la actualidad. Se dispuso, además, una serie control con gatos descerebrados, para comparar los resultados obtenidos en estos animales con los procedentes de aquéllos sometidos a la acción de las anestesias antes citadas, y, por último, se trató de extender la comparación a los resultados registrados en animales intactos mantenidos en la cámara de reflejos condicionados.

MÉTODO

Se hicieron 123 experimentos en 64 perros y 59 gatos. Los anestésicos que se estudiaron fueron: el uretano, en dosis de 1.25 g por kg de peso, administrado en solución al 25 % e inyectando una tercera parte por vía intravenosa y dos terceras partes intraperitoneal, empleado solamente en perros; la cloralosa Merck, en dosis de 100 miligramos por kg de peso, en forma de solución al 0.55 % en agua destilada, inyectada por vía endovenosa, en perros; el Dial "Ciba", en dosis de 0.7 cm^3 por kg de peso por vía intraperitoneal para los gatos, y de 0.65 cm^3 por kg de peso, también por vía intraperitoneal, para los perros; el nembutal "Abbott", en dosis de 55 mg por kg de peso, en solución al 3 %, por vía intraperitoneal, para gatos y de 40 mg por kg de peso, en solución al 3 % por la misma vía, para perros; el pentotal sódico "Abbott", en solución al 5 % de 0.04 g a 0.08 g por kg de peso, por vía endovenosa, solamente en perros.

Los animales utilizados fueron anestesiados exclusivamente con la substancia que era objeto de estudio, evitando así la complicación que significa aplicar una substancia durante la acción de otra, como en el caso de Gruber [22] que inyectaba distintos barbitúricos a animales anestesiados con éter, Vercauteren [69] que inyectaba distintas substancias a perros anestesiados con cloralosa, y otros autores, que producen una inducción previa con morfina. Esta misma objeción ya fué

hecha por Hafkesbring ([30]) y Maloney ([53]), demostrando éste último que la morfina deprime más profundamente el centro respiratorio en los animales barbiturizados.

Se registraron en el quimógrafo, de manera sistemática y en forma simultánea, presión arterial, respiración y electrocardiograma. El registro de la respiración se hizo por medio de una aguja introducida en la tráquea, o por un neumógrafo colocado en el límite tóraco-abdominal; en ambos casos se utilizó una cápsula inscriptora del tipo de Jackson. Para el registro de la presión arterial se tomaron a menudo ambas arterias carótidas que, después de canuladas, se unieron a dos manómetros, uno de mercurio y otro de membrana del tipo de Hürthle; la única medida anticoagulante que se tomó fué la de llenar con solución saturada de sulfato de magnesia el sistema de tubos que conectaba a los manómetros con las arterias. En los casos en que se hizo el aislamiento circulatorio de los senos carotídeos se canularon los cabos craneales de las carótidas

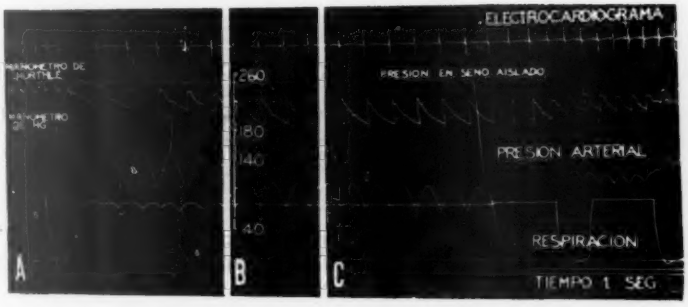

FIG. 1. — Perro. Cloralosa. Efectos que produce sobre corazón, presión arterial y respiración, el aumento brusco de la presión hidrostática (0 a 245 mm Hg) en el seno carotídeo aislado. Entre A y B, 11 segundos, entre B y C, 18 segundos.

comunes y se conectaron a una botella de presión que contenía suero fisiológico; en este sistema se intercaló un manómetro que inscribía las variaciones de presión estática a que eran sometidos los senos carotídeos. El electrocardiograma fué registrado con un aparato especialmente construído en nuestro laboratorio, que realiza la inscripción directa sobre el papel ahumado; como electrodos se utilizaron alfileres de seguridad insertados a través de la piel. Se usó la primera o la segunda derivación.

Para la excitación del cabo periférico del vago se empleó un circuito multivibrador que generaba ondas cuadradas. La frecuencia fué de 25 c.p.s. con una duración para cada estímulo de media sigma e intensidad máxima. Durante la excitación se mantuvo el control osciloscópico de la salida del estimulador.

El tipo de operación se procuró que fuera lo más uniforme posible y consistió, fundamentalmente, en disecar lo indispensable para llegar hasta el paquete cardiovascular del cuello y, en los casos necesarios, hasta la región del seno carotídeo. Para aislar los senos carotídeos se ligaban

las carótidas externa e interna, la occipital y la faríngea, así como también dos pequeños vasos que con gran constancia se encuentran en la región del glomus; estas manipulaciones se hacían con la máxima precaución para evitar en lo posible traumatizar la inervación del seno. En casi todos los experimentos se consiguió un aislamiento circulatorio perfecto, obteniéndose buenas respuestas reflejas a las variaciones de la presión hidrostática, en la bifurcación carotídea, durante períodos que alcanzaron hasta 7 h de duración; un ejemplo de estas respuestas se ilustra en la figura 1.

La descerebración se hizo con rapidez, bajo una ligera anestesia de éter y a través de una trepanación amplia, que se extendía desde la tienda del cerebelo hasta los senos frontales, abarcando ambos parietales. La sección cerebral se verificó entre los tubérculos cuadrigéminos anteriores y posteriores, con extirpación total de lo que queda por encima de este corte; las arterias carótidas permanecían ocluídas sólo durante el tiempo

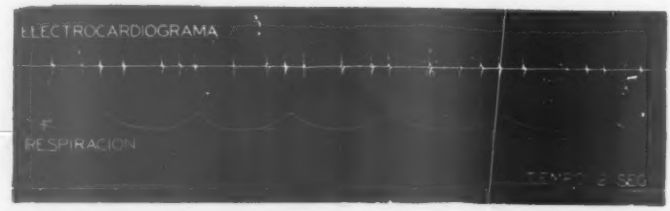

FIG. 2. — Perro. Sueño natural. Registros de electrocardiograma y respiración obtenidos en la cámara de reflejos condicionados.

de descerebración. Las observaciones se empezaban cuando el reflejo palpebral recuperaba su viveza normal; en principio esta preparación se asemeja mucho a las descritas por Izquierdo (42) y Stella (63).

En la cámara de reflejos condicionados (Alvarez-Buylla, 5), se hicieron registros de respiración y electrocardiograma en perros, durante el sueño natural y en estado de vigilia. Un ejemplo de estos registros se ilustra en la figura 2.

Fué necesario hacer un número suficiente de experimentos para obtener cifras medias que permitieran hacer las comparaciones deseadas. La elección de unidad comparativa también fué difícil, pues como ya indicó Hafkesbring (30) no se puede comparar la frecuencia cardíaca o el electrocardiograma del perro bajo la acción del pentotal con los registros del mismo animal antes de anestesiarlo. Aún en las condiciones especiales de aislamiento que proporciona la cámara de reflejos condicionados se observa gran variabilidad de la respiración y el electrocardiograma en el perro despierto; como por el contrario, durante el sueño natural, las frecuencias respiratoria y cardíaca son muy uniformes, se decidió tomar estas cifras como normales y utilizarlas como unidad de comparación.

RESULTADOS

I. — Comparación de la actividad cardíaca, de la presión arterial y la respiración en los animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano

1) Actividad Cardíaca.

a) Frecuencia. — Las cifras de la frecuencia cardíaca que presentan los perros y gatos, bajo la acción del dial, nembutal, pentotal, cloralosa y uretano, y su comparación con las obtenidas en gatos descerebrados y en perros intactos, durante el sueño natural y la vigilia proporcionan datos importantes para todo trabajo en que se empleen animales anestesiados con cualquiera de estas substancias y en los cuales se deseen estudiar las acciones nerviosas simpáticas y parasimpáticas o investigar la acción de distintas drogas.

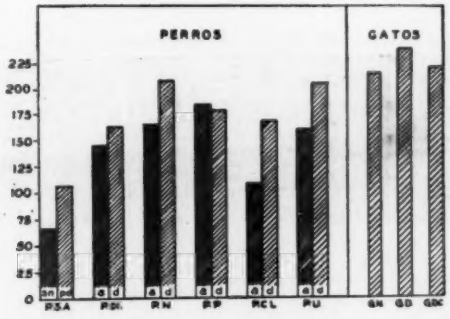

FIG. 3. — Cuadro comparativo de la frecuencia cardíaca en los animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano; gatos descerebrados y perros sin anestesiar, en estado de vigilia y durante el sueño natural. Ordenadas: Frecuencia cardíaca en ciclos por minuto. Abscisas: De izquierda a derecha: P.S.A. perros sin anestesiar; *s.n.* perros durante el sueño natural; *pd.* perros despiertos, pero aislados en la cámara de reflejos condicionados. P.Di., perros anestesiados con dial. P.N., perros anestesiados con nembutal. P.P., perros anestesiados con pentotal. P.Cl., perros anestesiados con cloralosa. P.U., perros anestesiados con uretano. Las columnas marcadas con *a* representan las cifras obtenidas antes de operar; las marcadas con *d* después de operar. G.N., gatos anestesiados con nembutal. G.D., gatos anestesiados con dial. G.DC., gatos descerebrados.

Para registrar la frecuencia cardíaca se escogió el método electrocardiográfico, por presentar ventajas definitivas sobre todos los demás empleados con estos fines, particularmente gracias a la adaptación que de él hemos hecho para la inscripción directa sobre papel ahumado, lo cual permite registrarlo simultáneamente con la presión arterial, y estudiar luego las relaciones de las manifestaciones eléctricas del corazón con los accidentes de la presión. Se hicieron siempre dos registros: uno cuando el animal estaba ya bajo los efectos de la anestesia, pero antes de la intervención quirúrgica, y otro al finalizar la intervención. Las cifras

obtenidas para cada anestésico, antes y después de operar, junto con las logradas en perros intactos, tanto durante el sueño natural como en estado de vigilia y en gatos descerebrados pueden compararse fácilmente en el cuadro de la figura 3.

El examen de este cuadro nos demuestra, en primer lugar, que existe diferencia entre la frecuencia cardíaca de los perros durante el sueño natural y el anestésico, ya que en el primero las cifras obtenidas oscilan entre 60 y 70 ciclos cardíacos por minuto, mientras que en los perros anestesiados la frecuencia está siempre por encima de 100.

La taquicardia más moderada se produce con la cloralosa y la más acentuada con el pentotal. Con todos los anestésicos, excepto con este último, se advierte que la intervención quirúrgica provoca, a su vez, una importante aceleración de la frecuencia cardíaca. En los gatos no se observan divergencias de frecuencia tan claras como en los perros. Las cifras en los gatos descerebrados y en los anestesiados con dial son algo más elevadas.

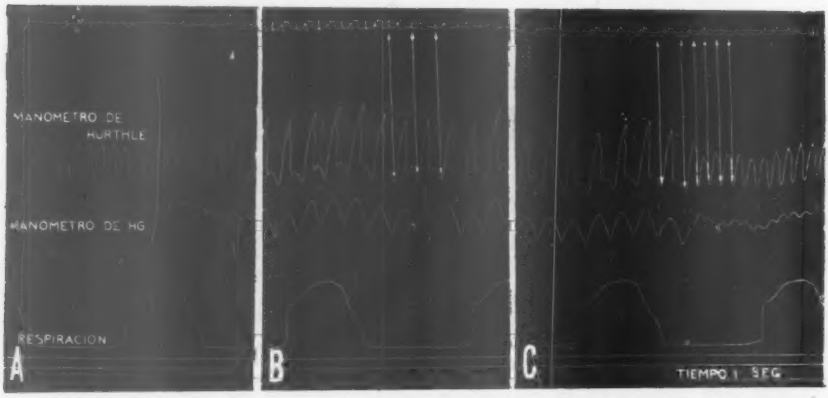

FIG. 4. — Perro. Pentotal. Aparición y desaparición del pulso bigeminado producido por las extrasístoles ventriculares, provocadas por la inyección endovenosa de 1 cm³ de pentotal sódico al 5 %, hecho 10 minutos antes de A; entre A y B, 48 segundos, entre B y C, 14 segundos. Las flechas señalan el fenómeno electrocardiográfico correspondiente en algunas ondas pulsatorias.

b) Deformaciones electrocardiográficas. — Se describen sólo aquellas deformaciones electrocardiográficas que aparecieron con más constancia y tuvieron un carácter más definido. Un minuto, aproximadamente, después de la inyección intravenosa de la dosis correspondiente de pentotal aparecen en el perro extrasístoles ventriculares que, por la constancia de su forma y relaciones temporales, hacen pensar que siempre tienen el mismo punto de partida; su efectividad en cuanto a volumen de sangre expulsado por el ventrículo es casi nula, por lo que sólo se advierte una pequeña muesca en el registro correspondiente del manómetro de membrana. La forma de aparición de este ritmo bigeminado

es repentina, dura unos 10 minutos y se recupera después totalmente y de manera instantánea (fig. 4).

En los perros anestesiados con nembutal se ha observado, con bastante frecuencia, una onda en el segmento T/P del electrocardiograma, que parece corresponder, en tiempo, con la descrita como onda U por Lewis [49]. Se ha comprobado que, temporalmente, corresponde a la iniciación de la onda dicrótica en el registro de la presión arterial, realizado con el manómetro de membrana (fig. 5). Los registros oscilográficos simultáneos del electrocardiograma y del neurograma del nervio depresor de Cyon y Ludwig confirmaron que esta onda coincide, efectivamente, con la inflexión que se ha denominado "diente dicrótico" (Karassek [48], Alvarez-Buylla [3-4], fig. 6).

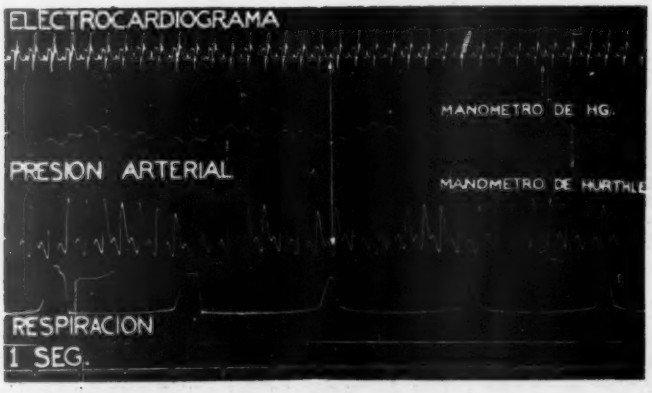

FIG. 5. — Perro. Nembutal. Onda U en electrocardiograma; las flechas señalan la correspondencia entre los fenómenos eléctricos del corazón y los trazos de presión arterial.

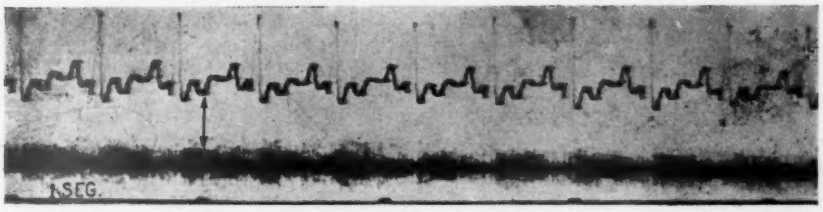

FIG. 6. — Perro. Nembutal. Registro simultáneo de electrocardiograma y neurograma del nervio depresor de Cyon y Ludwig; las flechas marcan la correspondencia entre los componentes del electrocardiograma y los del neurograma del nervio aórtico.

c) Efectos sobre la inhibición vagal. — Como se indica en la introducción de este trabajo, existen datos contradictorios acerca de la acción de los barbitúricos sobre las terminaciones efectoras del vago en el cora-

zón. Son muchos los autores que, utilizando barbitúricos como anestésico, han hecho estudios acerca de la influencia del vago sobre la frecuencia cardíaca (Rosenblueth y Simeone [60], Rosenblueth y Rioch [61], Walcott y Deyrup [72], Wang [71], y otros). Sin embargo, estos trabajos no se hubieran podido llevar a cabo si, como lo afirman algunos de los autores citados a este respecto en la introducción, las terminaciones efectoras del vago en el corazón quedaran bloqueadas por la acción de los barbitúricos, y las conclusiones a que se ha llegado en estos estudios sobre inervación vagal del corazón deberían contener serios errores si se realizaron en animales con barbitúricos y éstos inhiben la acción vagal. En el curso de este trabajo se ha podido comprobar que la vagotomía bilateral, hecha en

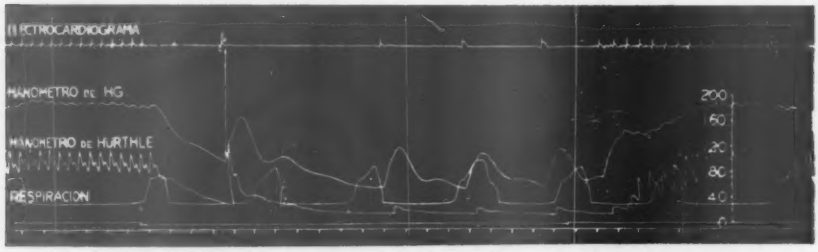

FIG. 7. — Perro. Nembutal. Excitación del cabo periférico del vago derecho. Paro cardíaco y escapes ventriculares.

perros bajo la acción de cada uno de los anestésicos aquí estudiados, ha producido siempre un aumento de frecuencia cardíaca. Esta taquicardia demuestra que, con todos los anestésicos, el vago continúa ejerciendo su acción tónica inhibidora. Los datos que se dan en la sección II, 1, demuestran que desde las zonas reflexogénicas senocarotídeas se pueden provocar sobre el corazón reflejos cuyo componente efector es el vago. Estos hechos indican que los anestésicos estudiados no paralizan las terminaciones vagales. Además, la excitación del cabo periférico del vago, en perros anestesiados con dial, nembutal, pentotal, cloralosa y uretano produce detención cardíaca, durante varios segundos, hasta que aparecen escapes ventriculares (fig. 7).

2) Presión arterial.

En la figura 8 se presenta un cuadro que permite comparar las cifras medias de presión arterial obtenidas en perros y gatos anestesiados con dial, nembutal, pentotal y cloralosa, y en el cual se puede observar la gran diferencia que existe entre los niveles medios de presión arterial observada durante la acción de cada uno de los anestésicos utilizados; el más alto nivel medio de presión arterial se observó en perros anestesiados con cloralosa y el más bajo en los que se utilizó uretano. En los gatos, el nivel más alto de presión arterial se obtuvo en animales descerebrados, siguiendo en elevación el de los anestesiados con nembutal y el más bajo el correspondiente a los que lo fueron con dial.

3) Frecuencia respiratoria.

En la figura 9 se dan a conocer las frecuencias respiratorias medias que se observaron en los animales sometidos a diferentes anestesias, así como las correspondientes a perros intactos, durante el sueño natural y en estado de vigilia, y a gatos descerebrados. Es interesante que el orden en que influyen los anestésicos sobre la frecuencia respiratoria es precisamente inverso al observado con relación a la presión arterial (figuras 8 y 9).

Este hecho viene a reforzar la idea de la íntima relación funcional existente entre los aparatos circulatorio y respiratorio que, según considera la escuela de Anogin (6) forman un solo sistema funcional, cuya finalidad es la respiración tisular (véase Alvarez-Buylla,[3, 4]). Desde este punto de vista resulta lógico pensar que la deficiencia funcional de uno de los componentes sea compensado por el otro; recientemente ha com-

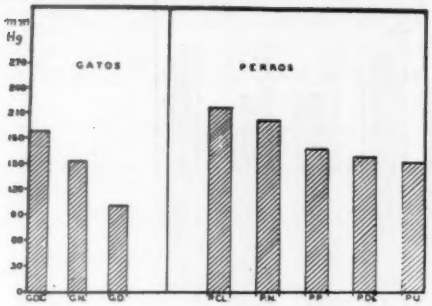

FIG. 8. — Cuadro comparativo de los niveles medios de presión arterial en animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano. Ordenadas: presión arterial en milímetros de mercurio. Abscisas: (de izquierda a derecha) G.DC., Gatos descerebrados; G.N., gatos nembutal; G.D., gatos dial; P.Cl., perros cloralosa; P.N., perros nembutal; P.P., perros pentotal; P.Di., perros dial; P.U., perros uretano.

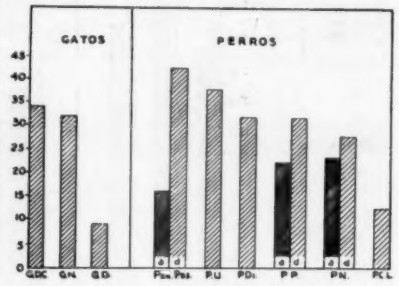

FIG. 9. — Cuadro comparativo de la frecuencia respiratoria media en animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano. Ordenadas: Frecuencia respiratoria en ciclos por minuto. Abscisas: G.DC., gatos descerebrados; G.N., gatos nembutal; G.D., gatos dial; P.sn., perros dormidos (sueño natural); P.ds., perros despiertos (en la cámara de reflejos condicionados); P.U., perros uretano; P. Di., perros dial; P.P., perros pentotal; P.N., perros nembutal; P.Cl., perros cloralosa. Las columnas marcadas con *a* representan las cifras obtenidas antes de operar, las marcadas con *d* después de operar.

probado Azpe (7) que los cambios respiratorios provocados por el uretano y por la cloralosa consisten en un aumento de la ventilación para el primero, y en una disminución de la misma para la segunda; la compensación sería lograda por disminución y por aumento correspondiente de la velocidad circulatoria. Más adelante presentamos pruebas de la realidad de estos cambios.

Debe hacerse notar, sin embargo, que en los gatos se observa bastante paralelismo entre los efectos sobre la presión arterial y sobre la

respiración (véanse las figuras 8 y 9). Lockhard ([52]) y Flinker ([19]) han hecho ver que existen diferencias en la forma de actuar de los anestésicos sobre perros y gatos.

II — Comparación de los efectos reflejos que se obtienen sobre el corazón, la presión arterial y la respiración al excitar una región senocarotídea de los animales anestesiados con dial, pentotal, cloralosa y uretano.

Para excitar los barorreceptores de la región senocarotídea se utilizó el método de Hering, pinzando la carótida común con objeto de producir una hipotensión brusca en la bifurcación de la misma. Para obtener ascensos bruscos se aisló circulatoriamente el seno carotídeo, como se describe en la parte correspondiente a método. Para juzgar la acción de los

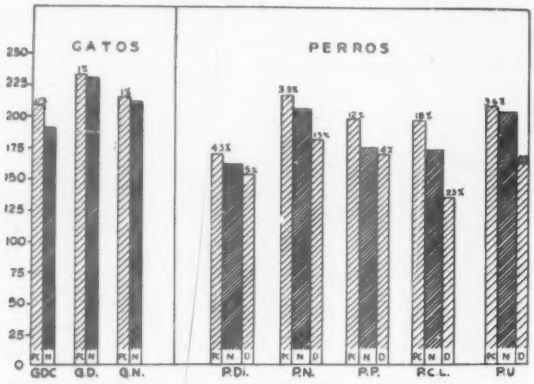

FIG. 10. — Cuadro comparativo de las modificaciones producidas, sobre la frecuencia cardíaca, por la hipotensión y la hipertensión provocadas en uno de los senos carotídeos, conservando intacta la inervación aórtica y la del otro seno.

Ordenadas: Ciclos cardíacos por minuto. Abscisas: Las columnas marcadas con *D* representan la frecuencia cardíaca durante la dilatación del seno carotídeo; las marcadas con P.C. representan la frecuencia obtenida durante el pinzamiento de la carótida común; las marcadas con N, representan la frecuencia obtenida antes de estas excitaciones. De izquierda a derecha: G.DC., gatos descerebrados; G.D., gatos dial; G.N., gatos nembutal; P.Di., perros dial; P.N., perros nembutal; P.P., perros pentotal; P.Cl., perros cloralosa; P.U., perros uretano.

anestésicos que nos ocupan, sobre los mecanismos reflejos reguladores de la presión, se tuvo en cuenta que la respuesta a la excitación de una zona reflexogénica determina cambios de presión en las otras zonas, que tienden a provocar cambios opuestos a los que produjo la primera zona excitada (Izquierdo, [43]); el anestésico que permita conservar una mayor eficacia en estos mecanismos reguladores será el que proporcione la respuesta mínima. Por esto fué necesario estudiar las respuestas que que se obtienen al excitar una de las regiones senocarotídeas, en las siguientes condiciones: *a)* cuando las restantes zonas reflexogénicas, cardioaórticas y el seno carotídeo opuesto quedan intactos, y *b)* después de la eliminación o interrupción de los caminos aferentes que las ponen en comunicación con el sistema nervioso central.

a) Comparación de los efectos reflejos sobre frecuencia cardíaca, presión arterial y respiración, que producen los cambios de presión en un seno carotídeo de animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano, conservando intacta la inervación de la aorta y la del otro seno carotídeo.

1. *Efectos sobre la frecuencia cardíaca.* — Para poner de manifiesto los efectos que sobre la frecuencia cardíaca producen el pinzamiento de la carótida y la dilatación de un seno aislado se contaron los ciclos cardíacos correspondientes a períodos de dos segundos y medio, durante todo el transcurso de la excitación, y con sus valores se trazó la curva correspondiente a cada experimento. El promedio de estas curvas es el que ilustra la figura 11, en la cual las abscisas representan el tiempo, (dos segundos y medio) y las ordenadas la frecuencia cardíaca en ciclos por minuto. El punto correspondiente al efecto máximo es el que aparece en el cuadro de la figura 10.

Este cuadro hace ver que en los perros anestesiados con cloralosa fué en los que se observó una mayor taquicardia al pinzar la carótida común y mayor bradicardia al dilatar un seno carotídeo aislado, siguién-

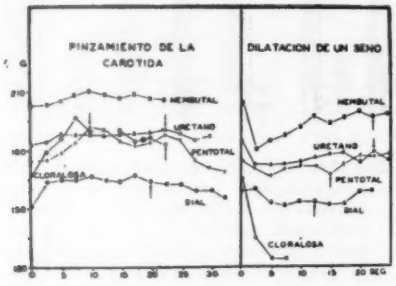

FIG. 11. — Curvas que representan el desarrollo temporal de los cambios de frecuencia cardíaca obtenida durante el pinzamiento de la carótida común y durante la dilatación de un seno carotídeo, en animales anestesiados con nembutal, dial, pentotal, cloralosa, uretano.

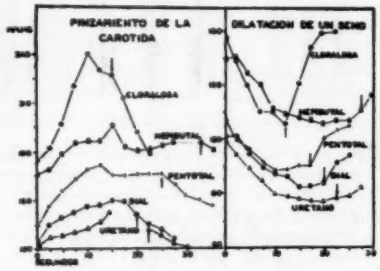

FIG. 12. — Curvas que representan el desarrollo temporal de los efectos hipertensores obtenidos durante el pinzamiento de la carótida común, y de los hipotensores producidos durante la dilatación de un seno carotídeo en animales anestesiados con: nembutal, dial, pentotal, cloralosa, y uretano.

dole en orden decreciente el nembutal, pentotal, uretano y dial. El efecto que produce la hipertensión en el seno carotídeo es mayor que el ocasionado por el pinzamiento de la carótida en los perros anestesiados con cloralosa, nembutal, uretano y dial; sólo con el pentotal se obtiene un efecto taquicárdico que excede en un 8 % al bradicárdico.

En los gatos descerebrados se logra un efecto taquicárdico claro durante el pinzamiento de la carótida común; sin embargo, en los anestesiados con dial y nembutal dicho efecto es insignificante, lo que puede deberse a que en estos animales la frecuencia cardíaca es ya más elevada

que la que se obtiene durante el pinzamiento de la carótida común en los gatos descerebrados.

Si se estudia cómo se desarrolla en el tiempo el efecto bradicárdico que produce la dilatación de un seno carotídeo aislado (figura 11) cuando se emplea cloralosa o nembutal, se verá una disminución casi inmediata de la frecuencia, efecto que es seguido a los pocos segundos por otro más moderado y alternante. Por medio de la vagotomía y la atropina puede comprobarse que el primer componente es vagal, mientras el segundo es simpático. En efecto, la bradicardia inicial, que tan claramente se observa con cloralosa, nembutal y uretano, se atribuye a excitación vagal, mientras los efectos tardíos, a menudo de carácter alter-

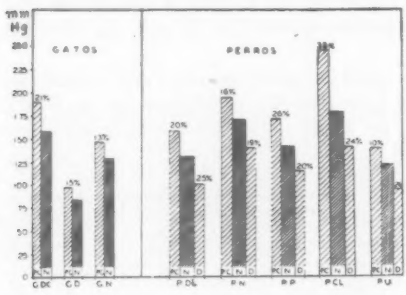

FIG. 13. — Cuadro comparativo de los cambios producidos en la presión arterial general, por los cambios locales de presión en uno de los senos carotídeos, conservando intacta la inervación aórtica y la del otro seno.

Ordenadas: presión arterial en mm Hg. Abscisas: Las columnas marcadas con *D*, representan la presión arterial durante la dilatación del seno carotídeo. Las marcadas con *P.C.* representan el cambio de presión obtenido durante el pinzamiento de la carótida común, y las marcadas con *N*, la presión arterial de los animales antes de estas excitaciones. De izquierda a derecha: G.DC., gatos descerebrados; G.D., gatos dial; G.N., gatos nembutal; P.Di., perros dial; P.N., perros nembutal; P.P., perros pentotal; P.Cl., perros cloralosa; P.U., perros uretano.

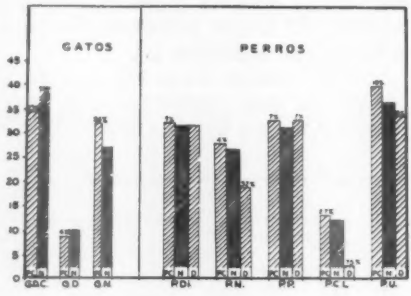

FIG. 14. — Cuadro comparativo de las modificaciones producidas sobre la respiración por la hipotensión y la hipertensión provocadas en uno de los senos carotídeos, conservando intacta la inervación aórtica y la del otro seno.

Ordenadas: respiración en ciclos por minuto. Abscisas: Las columnas marcadas con D representan la frecuencia respiratoria durante la dilatación del seno carotídeo. Las marcadas con P.C. representan la frecuencia obtenida durante el pinzamiento de la carótida común, y las marcadas con N la frecuencia respiratoria antes de estas dos excitaciones. De izquierda a derecha: G.DC., gatos descerebrados; G.D. gatos dial; G.N., gatos nembutal; P.Di., perros dial; P.N., perros nembutal; P.P., perros pentotal; P.Cl., perros cloralosa; P.U., perros uretano.

nante, son atribuídos a inhibición del tono del simpático. Por el contrario, la taquicardia que se obtiene como respuesta en el pinzamiento de la carótida se atribuye al efector simpático.

2. *Efectos sobre la presión arterial.* — Para poner de manifiesto los efectos que sobre la presión arterial producen el pinzamiento de la caró-

tida y la dilatación de un seno aislado se midió el nivel de la presión arterial cada dos segundos y medio, durante todo el transcurso de la excitación, y con los valores obtenidos se trazó la curva correspondiente a cada experimento. El promedio de todas las curvas es el que puede verse en la figura 12.

En la figura 13 se expresan, en forma de cuadro, los valores absolutos, así como el porcentaje de las respuestas (tanto alzas como bajas) obtenidas sobre la presión arterial en animales bajo la acción del dial, nembutal, pentotal, cloralosa y uretano. Las respuestas mayores las encontramos en los perros anestesiados con cloralosa, figurando a continuación las debidas al nembutal, pentotal y dial, siendo las menores las provocadas por el uretano. Si comparamos la magnitud de las respuestas hiper o hipotensoras con cada anestésico, veremos que con la cloralosa y con el pentotal es mayor la respuesta al pinzamiento de la carótida común que a la dilatación del seno, mientras que con uretano, dial y nembutal, domina el descenso de la presión arterial, obtenido como respuesta a la dilatación del seno aislado. El pinzamiento de la carótida es más manifiesto en los gatos descerebrados que en los anestesiados con nembutal, siendo la respuesta correspondiente a este último anestésico más intensa que la obtenida con el dial. En la figura 12 se presentan las curvas medias de los efectos obtenidos con cada uno de los anestésicos al excitar una región senocarotídea. En esta figura y en la 13 se puede comprobar que, ordenando los anestésicos según la magnitud de las respuestas, se obtiene el mismo resultado que al disponerlos según la media de presión

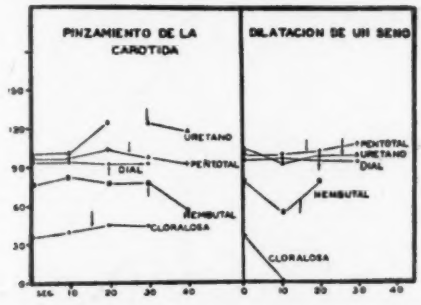

FIG. 15 — Curvas que representan el desarrollo temporal de los efectos respiratorios obtenidos durante el reflejo de Hering y durante la dilatación de un seno carotídeo, en animales anestesiados con nembutal, dial, pentotal, cloralosa, y uretano.

arterial. Por lo tanto, en ambos casos el primer lugar lo ocupa la cloralosa, con la cual se observan efectos consistentes en un ascenso notable seguido de un descenso brusco, como puede verse en la figura 12. Estos efectos con la cloralosa contrastan con la suavidad de las curvas correspondientes al dial y al uretano.

3. *Efecto sobre respiración.* — Para el análisis de los efectos sobre la respiración se contaron los movimientos cada 10 segundos, en lugar de cada dos segundos y medio como para la presión arterial y la frecuen-

cia cardíaca. Iguales procedimientos se siguieron para la construcción de cuadros y curvas, que los utilizados para analizar la presión arterial y frecuencia cardíaca.

Como se demuestra en el cuadro de la figura 14, es en los animales anestesiados con cloralosa, nembutal y uretano, en los que se obtienen los efectos más constantes y manifiestos sobre la frecuencia respiratoria, al pinzar la carótida común o al dilatar un seno carotídeo aislado. La frecuencia respiratoria es afectada apenas en los animales anestesiados con dial. En los gatos descerebrados el pinzamiento de la carótida se manifiesta en forma de una clara bradipnea. En los anestesiados con dial el pinzamiento de la carótida también provoca bradipnea, aunque insignificante. Por el contrario, dicho reflejo ejerce una evidente influencia aceleradora sobre la frecuencia respiratoria en los gatos anestesiados con nembutal. También tuvo carácter paradójico la taquipnea obtenida en los perros anestesiados con pentotal al dilatar un seno carotídeo. En las curvas que se presentan en la figura 15 puede verse lo poco marcados e inconstantes de los efectos que producen la excitación de los barorreceptores de las zonas reflexogénicas sobre el ritmo respiratorio.

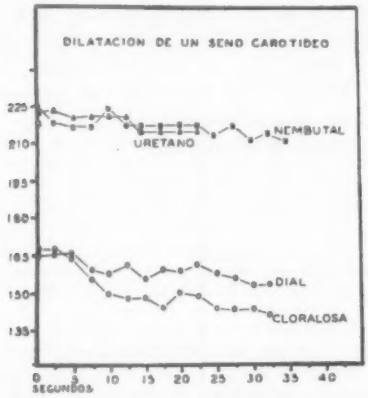

FIG. 16. — Desarrollo temporal de las respuestas obtenidas sobre la frecuencia cardíaca en perros anestesiados con dial, nembutal, cloralosa y uretano, al dilatar un seno carotídeo aislado después de la extirpación del otro. Ordenadas: ciclos cardíacos por minuto. Abscisas: Tiempo.

FIG. 17. — Desarrollo temporal de las respuestas obtenidas sobre la presión arterial en perros anestesiados con dial, nembutal, cloralosa y uretano.

III — Estudio comparativo de las respuestas obtenidas sobre la frecuencia cardíaca, presión arterial y respiración al dilatar un seno carotídeo aislado después de la extirpación del otro y de la vagotomía bilateral, en perros anestesiados con dial, nembutal, pentotal, cloralosa y uretano.

Realizada la preparación indispensable para aislar uno de los senos carotídeos y hacer la resección del otro, se seccionaban ambos vagos. Una vez estabilizados los registros después de los disturbios que tienen lugar como consecuencia de estas manipulaciones, se procedió a dilatar el seno aislado, estudiándose los efectos que esta dilatación producía sobre la frecuencia cardíaca, presión arterial y frecuencia respiratoria.

1. *Efecto sobre frecuencia cardíaca.* — Como después de la administración de los anestésicos estudiados, la vagotomía bilateral ocasiona aumento de la frecuencia cardíaca, es claro que el tono vagal cardíaco no es eliminado por aquéllos. Sin embargo, importa hacer notar que semejante taquicardia desaparece por lo general en el término de 1 ó 2 horas.

La dilatación de un seno con los dos vagos seccionados produce sobre la frecuencia del corazón los efectos que pueden verse en la figura 16, (atribuibles a reflejos del simpático, Herman [34], Winder [73]). Comparados con los efectos vagales, ofrecen en general mayor latencia y carácter alternante.

También en este caso es bajo la acción de la cloralosa cuando se observan los reflejos más claros, y con el uretano cuando son menos marcados. Hay que advertir que los valores con que se construyeron estas

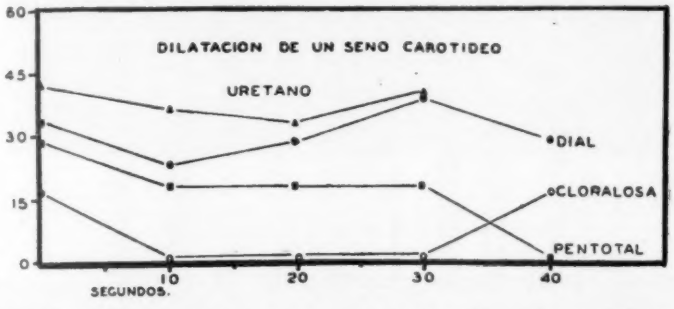

FIG. 18. — Desarrollo temporal de las respuestas obtenidas sobre frecuencia respiratoria en perros anestesiados con dial, pentotal, cloralosa y uretano al dilatar un seno carotídeo aislado después de la extirpación del otro y de la vagotomía bilateral. Ordenadas: respiración en ciclos por minuto. Abscisas: Tiempo.

curvas son promedios de distintos experimentos, pero que en algunos de ellos se obtuvieron efectos simpáticos más notables, incluso con el uretano.

2. *Efectos sobre presión arterial.* — En la figura 17 se pueden comparar los efectos obtenidos sobre la presión arterial por la dilatación de un seno carotídeo aislado en perros a los cuales se les había resecado el otro seno, hecho la vagotomía bilateral y administrado distintos anestésicos. Si se comparan estas curvas con las de la figura 12, se ve que, con todos los anestésicos, el efecto resulta aumentado aproximadamente en un 50 %. Con el dial y el uretano la forma de las curvas apenas si indica un cambio; con la cloralosa y el nembutal la parte inicial de las

curvas aparece muy desfigurada (véanse las figuras 12 y 16) quizá porque con estos anestésicos el componente vagal es más importante y por lo tanto influye más intensamente sobre la parte inicial del trazo. Igual influencia se pone de manifiesto por el aumento de latencia en las curvas de la cloralosa y del nembutal, después de eliminar los vagos. Por el contrario, la parte inicial de las curvas correspondientes al uretano y al dial no se modifica.

3. *Efecto sobre la frecuencia respiratoria.* — La vagotomía y la resección de un seno producen una clara disminución de su frecuencia. Sus modificaciones reflejas por dilatación de un seno carotídeo aislado, según puede verse en la figura 18, son semejantes a las que ilustra la figura 15. Los efectos más claros también corresponden a los animales anestesiados con cloralosa y nembutal.

IV. — Efectos que produce la deaferentación total de las zonas reflexogénicas senocarotídeas y cardioaórtica en los perros anestesiados con dial, nembutal, pentotal, cloralosa, uretano y en gatos descerebrados o anestesiados con dial o nembutal.

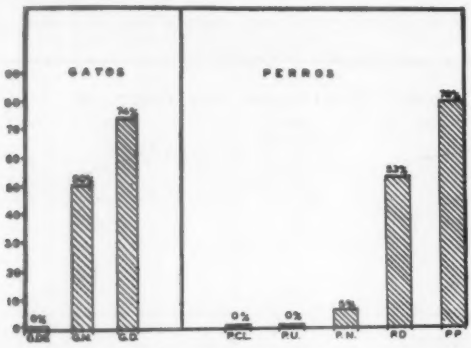

FIG. 19. — Cuadro comparativo del porcentaje de accidentes mortales ocurridos durante la deaferentación reflexogénica en animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano

Muchos autores: Hering (33), Koch (46), Heymans (35), Dautrebande (16), Nowak (55), Witt, Katz y Kohn (74) han observado que la extirpación de las zonas reflexogénicas senocarotídeas y cardioaórticas produce hipertensión crónica. Sin embargo, se ha dedicado muy poca atención a los efectos inmediatos resultantes de la deaferentación total de dichas zonas, así como a las influencias que los anestésicos pueden tener sobre tales resultados.

Todos los autores están de acuerdo en que, durante la eliminación de los controles reflexogénicos, hay un gran número de accidentes mortales, y según Witt, Katz y Kohn (74) los anestésicos podrían tener intervención importante en estos accidentes. Con el afán de poner en claro tales hechos empezamos por determinar el porcentaje de muertes regis-

tradas con cada uno de los anestésicos al intentar la resección de ambos senos y la vagotomía bilateral (véase cuadro de la figura 19). Encontramos que no hubo accidentes mortales con el uretano ni con la cloralosa, que murió el 5 % de los perros con el nembutal, y el 50 % con dial. Los gatos descerebrados sobrevivieron en su totalidad la deaferentación reflexogénica. De los anestesiados con nembutal murió el 50 %; con dial, el 74 %.

Las muertes sobrevienen por depresión respiratoria progresiva que pronto va seguida por la de la función vasomotora (figuras 20 y 21), tal como lo tienen observado Witt, Katz y Kohn (74), Beecher (9).

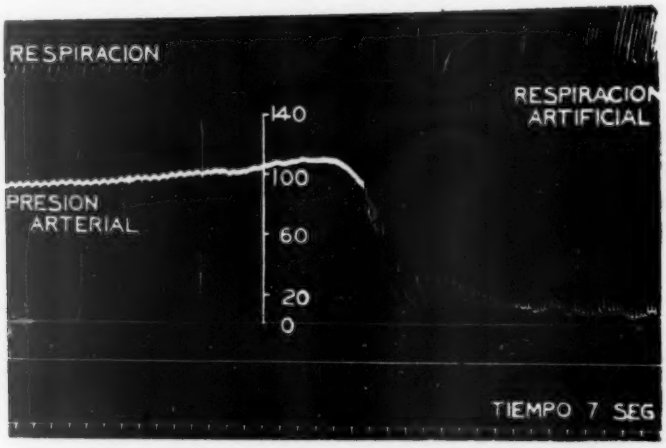

FIG. 20. — Gato. Dial. Depresión respiratoria y vasomotora consecutiva a la resección de los dos senos carotídeos después de la vagotomía bilateral. La respiración artificial no detuvo el descenso de la presión, y el animal murió.

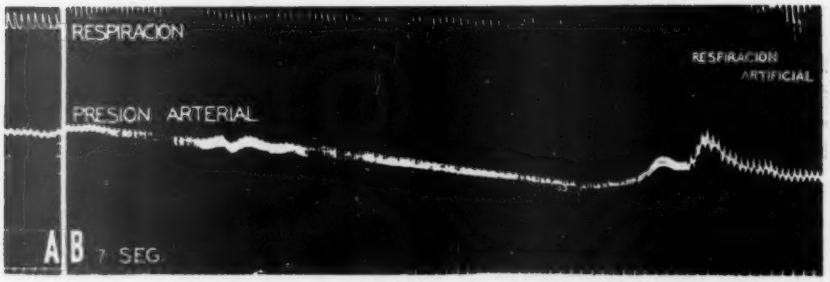

FIG. 21. — Gato. Dial. Depresión respiratoria y vasomotora más lenta que la ilustrada en la figura 21. Entre A y B, deaferentación reflexogénica. Con respiración artificial se consiguió mantener la presión a 60 mm Hg durante varias horas. En este caso el establecimiento más oportuno logró mantener la presión arterial aunque ya no se obtienen las respuestas del seno.

Para determinar la influencia de la vagotomía bilateral y de la resección de ambos senos carotídeos sobre la frecuencia cardíaca, la presión arterial y la respiración de los animales que sobrevivieron a la deaferentación, se hizo la eliminación de ambos senos carotídeos, siguiendo el método antes indicado, y además la vagotomía bilateral. Antes de hacer las medidas se esperó hasta que la frecuencia cardíaca, la presión arterial y la frecuencia respiratoria se hubieran estabilizado.

1. *Efecto sobre la frecuencia cardíaca.* — En los perros anestesiados

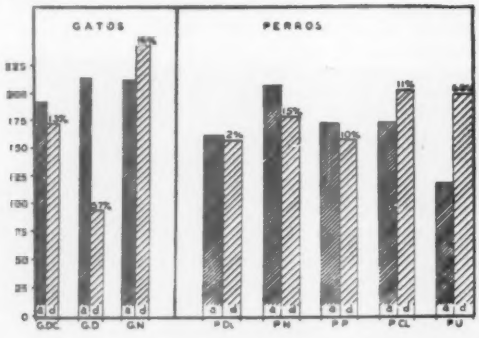

FIG. 22. — Cuadro comparativo de los cambios de frecuencia cardíaca en los animales que sobrevivieron a la deaferentación reflexogénica, anestesiados con dial, nembutal, pentotal, cloralosa y uretano. Ordenadas: Ciclos cardíacos por minuto. Abscisas: De izquierda a derecha: G.D.C., gatos descerebrados; G.D., gatos dial; G.N., gatos nembutal; P.Di, perros dial; P.N., perros nembutal; P.P., perros pentotal; P.Cl., perros cloralosa; P.U., perros uretano. Las columnas marcadas con *a* representan las frecuencias iniciales, las marcadas con *d* representan sus cambios después de deaferentar.

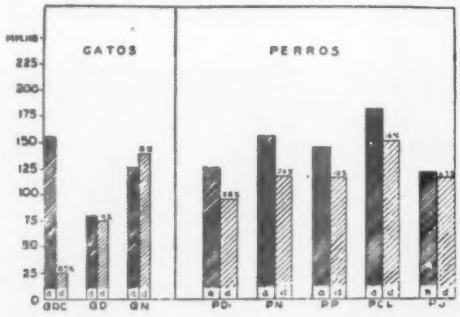

FIG. 23. — Cuadro comparativo de los cambios de presión arterial en animales que sobrevivieron la deaferentación reflexogénica, anestesiados con dial, nembutal, pentotal, cloralosa y uretano. Ordenadas: presión arterial en mm Hg. Abscisas: G.D.C., gatos descerebrados; G.D., gatos dial; G.N., gatos nembutal; P.Di., perros dial; P.N., perros nembutal; P.P., perros pentotal; P.Cl., perros claralosa; P.U., perros uretano. Las columnas marcadas con *a* representan los valores antes de deaferentar; las marcadas con *d* indican los subsiguientes.

con uretano y cloralosa, la vagotomía y la resección de ambas zonas senocarotídeas provocan una taquicardia importante y duradera. En los perros con dial la frecuencia cardíaca no varía, y en los anestesiados con nembutal y pentotal termina por estabilizarse a una frecuencia menor que la inicial. La figura 22 permite comparar las frecuencias cardíacas antes y después de eliminar los controles reflexogénicos.

2. *Efectos sobre la presión arterial.* — Con todos los anestésicos estudiados la deaferentación total determinó estabilización de la presión arterial a nivel más bajo que el inicial.

Es verdad que en el momento de anular tal o cual unidad reflexogénica se observan frecuentemente taquicardias e hipertensiones más o menos bruscas, pero éstas casi siempre son pasajeras y en el término de 1 a 4 horas se estabiliza la presión arterial a los niveles que se indican

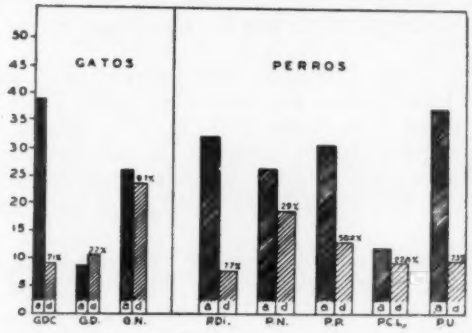

FIG. 24. — Cuadro comparativo de los cambios de respiración en animales que sobrevivieron la deaferentación reflexogénica anestesiados con dial, nembutal, pentotal, cloralosa y uretano. Ordenadas: ciclos respiratorios por minuto. Abscisas: G.D.C., gatos descerebrados; G.D., gatos dial; G.N., gatos nembutal; P.Di., perros dial; P.N., perros nembutal; P.Cl., perros cloralosa; P.U., perros uretano; P.P., perros pentotal.

en el cuadro que aparece en la figura 23, cuyos datos concuerdan con las observaciones de Hwang Kao y sus colaboradores (41).

3. *Efectos sobre la respiración.* — Los efectos de la vagotomía bilateral y la resección de los dos senos carotídeos produjeron, con todos los anestésicos, una marcada disminución de la frecuencia respiratoria, según puede verse por el cuadro de la figura 24, que consigna las frecuencias respiratorias antes y después de eliminar los dos senos carotídeos y ambos vagos. Sin embargo, la depresión es ligeramente mayor con los anestésicos que provocan mayores frecuencias respiratorias, disminuyendo así las diferencias antes observadas (compárense la fig. 9 con la 24).

DISCUSIÓN

El registro del electrocardiograma, hecho directamente sobre el quimógrafo, resultó muy útil y cómodo, pues además de proporcionar un control inmejorable del ritmo cardíaco, sin el menor traumatismo, hizo

posible el estudio simultáneo de los accidentes eléctricos que produce la contracción cardíaca con sus efectos mecánicos sobre presión arterial. Por este procedimiento, la comparación temporal de los fenómenos que se registran en el electrocardiograma, presión arterial y respiración, se hace con mayor facilidad y precisión que obteniendo una inscripción separada del electrocardiograma. Las extrasístoles pueden verse fácilmente, evitándose errores como el de tomar por bradicardia lo que en realidad es pulso bigeminado producido por extrasístoles ventriculares que se alternan con contracciones normales, como sucede con el pentotal, cuyo efecto pasó inadvertido para Betlach (11). Las afirmaciones de Gruber sobre la dependencia de estas extrasístoles ventriculares del nivel de presión arterial, no se han confirmado en nuestras experiencias, ni tampoco que puedan ser influídas por las zonas senocarotídeas. Como las extrasístoles ventriculares desaparecen o surgen por igual, al pinzar las carótidas o al dilatar el seno carotídeo aislado, surgiendo a menudo espontáneamente, puede pensarse que las observaciones de Gruber fueron coincidencias casuales.

En cuanto a la onda intermedia que aparece en el electrocardiograma de los perros anestesiados con nembutal, entre T y P, y que quizá corresponda a la onda U de Lewis (49), lo único que se puede afirmar es que aparece en el momento de cierre de las válvulas semilunares aórticas, ya que coincide exactamente con la onda dicrótica, tanto en el registro del manómetro de Hürthle (fig. 5) como en el neurograma del nervio depresor (fig. 6).

En uno de los perros anestesiados con cloralosa se produjeron extrasístoles ventriculares muy semejantes a las observadas en los perros anestesiados con pentotal.

Los anestésicos estudiados dan una frecuencia cardíaca mucho más alta que la correspondiente a los perros durante el sueño natural. Esta aceleración de la frecuencia cardíaca, que experimentan los perros como consecuencia de la anestesia, es cuestión que no puede darse por solucionada con las afirmaciones de Gruber (22), Koppany, Linegar y Dale (45), Gruber, Haury y Gruber (27), Gruber, Haury y Gruber (23), Gruber, Freeman y Keyser (25), Gruber, Gruber y Colosi (26), Linegar, Dille y Koppany (51), Swanson (68), Garry (20), Lieb y Mulinos (50); Schaefer y Underwood (65), quienes sostienen que los barbitúricos paralizan el vago cardíaco.

De los hechos y argumentos presentados aquí no se puede deducir si los barbitúricos ejercen o no alguna acción inhibidora sobre las terminaciones periféricas del vago, pero se puede afirmar que estas terminaciones vagales del corazón no llegan a ser paralizadas, ya que con todos los anestésicos estudiados en este trabajo, se consiguió detener el corazón durante varios segundos, excitando el cabo periférico del vago, y obtener efectos taquicárdicos o bradicárdicos al excitar las zonas reflexogénicas. La intervención del vago en estas reacciones se pone claramente de manifiesto utilizando atropina o practicando la vagotomía bilateral. Los hechos presentados vienen a reforzar las afirmaciones hechas por Krayer (46) de que los barbitúricos actúan directamente sobre el miocardio.

En la sección II nos limitamos al estudio fenomenológico, sin intentar la explicación de las causas que motivan las diferencias obtenidas según el anestésico empleado, ni las que se aprecian en las respuestas de los mecanismos propioceptivos reguladores de la circulación sanguínea en animales anestesiados con distintas substancias, por creer que los hechos son insuficientes para ello y porque la finalidad de este trabajo se limita a proporcionar los datos que puedan servir de ayuda en el momento de decidir la selección de anestésico. Sin embargo, quisiéramos hacer notar que el aumento de las respuestas de la presión arterial observado al excitar un seno carotídeo después de eliminar el otro y seccionar ambos vagos, no tiene carácter constante, es decir, que este aumento sufre variaciones en el tiempo. Hay que pensar, por lo tanto, que en él intervienen dos factores: *a)* la eliminación de los efectos "amortiguadores" que se originan en la zona cardioaórtica y senocarotídea (factor que sería de valor constante) y *b)* una cierta hipersensibilidad central cuya naturaleza correspondería a la de los fenómenos de hipersensibilidad por denervación, descritos por Cannon y Rosenblueth [14]; este segundo factor estaría, por lo tanto, sujeto a cambios en el tiempo similares a los descritos por dichos autores.

A pesar de existir un número considerable de experiencias con resultados negativos, muchos fisiólogos sostienen que la resección de los senos carotídeos y de la inervación aferente de la zona cardioaórtica producen un estado de hipertensión crónica.

Cuando la hipertensión no se obtiene, según Heymans y Bouckaert [37] es consecuencia de errores técnicos en la operación, debidos a que se conserva alguna fibra nerviosa que más tarde compensa la función de los controles eliminados. Sin embargo, en trabajos relativamente recientes, como el de Green [21], donde se tomaron toda clase de precauciones para evitar este tipo de errores técnicos, las hipertensiones y taquicardias obtenidas fueron transitorias.

Witt, Katz y Kohn [74] afirman que después de la deaferentación total obtuvieron en muchas ocasiones hipotensión en lugar de hipertensiones, señalando la posibilidad de ser el anestésico el responsable.

En nuestros experimentos se obtuvo hipertensión como consecuencia de la deaferentación reflexogénica, pero siempre fué pasajera, siendo su duración máxima 1 h y estabilizándose la presión a niveles siempre más bajos que los que tenían los animales antes de la deaferentación. La taquicardia que se obtiene después de esta deaferentación es mucho más estable que la hipertensión, pero, al igual que ésta, disminuye más o menos paulatinamente. Al cabo de 4 ó 5 h de observación la frecuencia cardíaca suele ser todavía algo más alta que antes de la deaferentación. Resultados semejantes aparecen en el trabajo de Hwang Kao, Essex y Mann [41].

Tales hechos se repitieron con todos los anestésicos estudiados, advirtiéndose que las reacciones transitorias de hipertensión y taquicardia fueron más acentuadas cuando el anestésico empleado fué cloralosa y las menos cuando se empleó dial. Con el uretano, la taquicardia fué más duradera.

El carácter transitorio de la hipertensión y la taquicardia que siguen a la deaferentación reflexogénica con todos los anestésicos estudiados, induce a pensar que no son estas substancias las responsables de este carácter transitorio, sino que existen mecanismos fisiológicos que compensan funcionalmente la eliminación de los reflejos cardioaórticos y senocarotídeos. Heyman (38) supone la existencia de zonas vasosensibles al final de la aorta torácica y principio de la abdominal. Hollinshead (40) afirma que existen quimiorreceptores abdominales.

Del grupo de anestésicos estudiados en este trabajo, la conclusión más clara resultante es la relativa al pentotal. Este tiobarbiturato es indudablemente el peor anestésico para estudios experimentales, por dar con mayor frecuencia accidentes respiratorios, por ser más difíciles de controlar los efectos tóxicos que ocasiona, ya que su toxicidad depende de la velocidad de la inyección, y del número de ellas que se hagan, y, por fin, su rápida destrucción en el organismo da lugar a una inestabilidad del nivel anestésico sometiendo a todos los mecanismos fisiológicos a altas y bajas que hacen más difícil su estudio. Estas conclusiones están de acuerdo con las observaciones de Reynolds (59), Draper (17) y Gruber (22). En cuanto al resto de los anestésicos, todos pueden ser útiles, según la finalidad que se persiga. El nembutal se prefiere para la deaferentación reflexogénica, por ser de aplicación cómoda y producir una anestesia duradera que permite ir eliminando los controles reflexogénicos con intervalos de 20 a 30 minutos, consiguiéndose disminuir la brusquedad de los cambios de presión arterial y frecuencia cardíaca.

La cloralosa es el anestésico con el cual los reflejos propioceptivos reguladores de la presión arterial presentan mayores reacciones. El uretano proporciona gran estabilidad vasomotora y el dial produce anestesias más estables y duraderas.

Resumen

1. — Se hicieron 123 experimentos en animales anestesiados con dial, nembutal, pentotal, cloralosa y uretano, comparando la influencia que ejercen estos anestésicos sobre electrocardiograma, presión arterial y respiración.

2. — El estudio se basó, fundamentalmente, en el análisis de registros simultáneos sobre papel ahumado, de presión arterial, respiración y electrocardiograma, gracias esto último a un dispositivo ideado por el autor.

3. — Se discute la utilidad de los datos obtenidos en perros durante la vigilia, durante el sueño natural y durante su permanencia en la cámara de reflejos condicionados, como término de comparación.

4. — Se presentan y comparan los datos obtenidos en gatos anestesiados con dial y nembutal, con los logrados en gatos descerebrados.

5. — Se describen los fenómenos electrocardiográficos característicos del pentotal y nembutal.

6. — En uno de los perros anestesiados con cloralosa se registraron extrasístoles ventriculares, semejantes a las producidas por el pentotal, pero en los demás anestesiados con cloralosa, o en los anestesiados con

uretano o dial, no se advirtieron deformaciones electrocardiográficas atribuibles a los mismos.

7. — Se comprobó que las terminaciones efectoras del vago en el corazón no son paralizadas por ninguno de los anestésicos estudiados.

8. — Los reflejos propioceptivos reguladores de la presión arterial se presentaron más notablemente con la cloralosa que con los otros anestésicos.

9. — La hipertensión y la taquicardia provocadas por la deaferentación reflexogénica son transitorias, sea cual fuere el anestésico empleado.

10. — De las experiencias del autor resulta que el nembutal es un anestésico muy útil y cómodo para la experimentación, siendo el menos aconsejable el pentotal.

El autor agradece al Dr. J. J. Izquierdo las correcciones hechas al manuscrito de este trabajo.

SUMMARY

The study was carried out on 64 dogs and 59 cats. The results in the anesthetized animals were compared with those obtained in intact dogs, during normal sleep (fig. 2), and in decerebrate cats.

Pentothal and nembutal cause electrocardiographic abnormalities (figs. 4, 5 and 6).

The tonic discharge of the vagal fibers that distribute to the heart is not abolished by any of the anesthetics tested: dial, nembutal, pentothal, chloralose and urethane. The vagal efferent pathway of the carotid sinus reflexes, likewise, is not interrupted. Peripheral stimulation of the cut vagus caused a marked slowing of the heart beat in all instances (figs. 1, 7, 10 and 11).

The mean blood pressure (fig. 8), heart rate (fig. 3), and respiratory rate (fig. 9), was determined for each of the anesthetics. The mean amplitude of the reflex changes of blood pressure, respiratory rate and heart rate, consequent to the stimulation of the baroreceptors of a carotid sinus, was also studied. These observations were made in the following experimental conditions: *a*) with the other carotid sinus and both vagi intact (figs. 11 to 15); *b*) after resection of a carotid sinus and bilateral vagotomy (figs. 16 to 18). In the latter conditions a reflex change of heart rate was detected, mediated by the sympathetic nerves.

Finally, the mortality due to resection of both carotid sinus regions and to bilateral vagotomy was compared for the different anesthetics (fig. 19). In the animals that survived this operations the hypertension and tachycardia were transient.

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Las medidas y símbolos deben expresarse de acuerdo con las recomendaciones de la Comisión de Símbolos, Unidades y Nomenclatura de la Unión Internacional de Física, aprobadas en Amsterdam, en julio 1948 *(Ciencia e Invest.*, 1949, *5*, 433).

Se exponen a continuación algunas abreviaturas comunes:

metro	m	litro	l	microgramo	µg
centímetro	cm	centímetro cúbico	cm^3	gamma	γ
milímetro	mm	mililitro	ml	por ciento	%
micrón	µ	kilogramo	kg	hora	h
milimicrón	mµ	gramo	g	minuto	m
Angström	Å	miligramo	mg	segundo	s
				milisegundo	ms

Para evitar la confusión derivada de la notación decimal diferente según los países, se adopta el punto decimal y se suprime toda notación entre millares sustituyéndose por un espacio: 10 000 (no 10.000 ni 10,000) — 0.90 (no 0,90).

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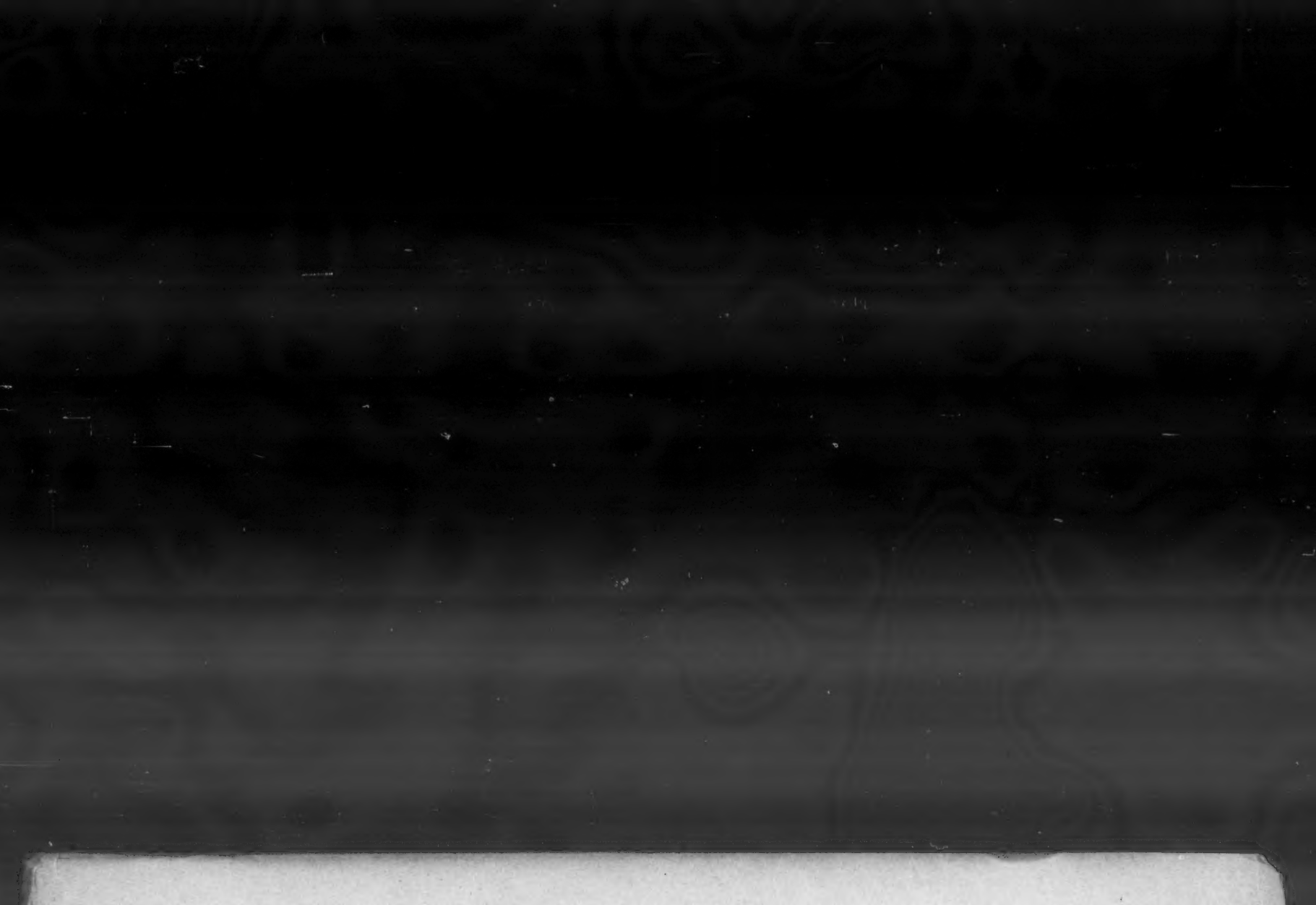
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